Controversies in Haematopoietic Stem Cell Transplantation

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Abstract

Progress in the understanding and practice of haematopoietic stem cell transplantation has come a long way since its inception. Despite this, there is ever-increasing controversy within most haematological malignancies about the role and timing of transplantation. Medical practice today is governed by óevidence-based medicineô and guidelines. However, guidelines are generic, with little quality evidence base available in many scenarios to help clinicians make decisions about transplantation in patients who fall into those controversial categories.

The morbidity and mortality associated with allogeneic transplantation remains significant. Autologous transplantation is a safer procedure but does not offer the same curative potential. Consequently, decisions about which patients should be transplanted, how and when, continue to cause controversy.

I have identified four clinical scenarios within haematopoietic stem cell transplantation where guidelines are not clear about the specifics of practice but in which we have specific clinical experience at St Bartholomewôs. These are the outcomes of two melphalan conditioning doses in autologous stem cell transplantation in multiple myeloma, the outcomes of allogeneic stem cell transplantation in multiple myeloma, the outcomes of patients with refractory and relapsed acute myeloid leukaemia and myelodysplasia undergoing sequential transplantation and finally the outcomes of allogeneic stem cell transplantation in lymphoma.

The aim of this thesis has been to collate and analyse patient data in each of these areas of debate, in order to make recommendations regarding future clinical practice. Following on from this, I have evaluated the role of haematopoietic stem cell transplantation today and its future directions.

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ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
AP	Accelerated phase
ASCT	Autologous stem cell transplant
ATG	Anti-thymocyte globulin
BMI	Body mass index
BP	Blast phase
BSA	Body surface area
BSBMT	British Society of Blood and Marrow Transplantation
CIBMTR	Centre for International Blood and Marrow Transplant Research
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CMV	Cytomegalovirus
CR	Complete remission/ complete response
CSA	Ciclosporin
DLA	Dog leucocyte antigen
DLBCL	Diffuse large B cell lymphoma
DLI	Donor lymphocyte infusion
EBMT	European Group for Blood and Marrow Transplantation
FDC	Full donor chimerism
FISH	Fluorescent in situ hybridisation
FL	Follicular lymphoma
FLIPI	Follicular lymphoma International Prognostic Index
FLT3	Fms-related tyrosine kinase 3
FTCC	Full T cell chimerism
GCSF	Granulocyte colony stimulating factor
GvH	Graft versus host
GVHD	Graft versus host disease
GvL	Graft versus leukaemia
HCT-CI	Haematopoietic cell transplant comorbidity index
HL	Hodgkin lymphoma
HLA	Human leucocyte antigen
HSCT	Haematopoietic stem cell transplant
IBMTR	International Blood and Marrow Transplant Research

IMWG	**International myeloma working group**
IPSS	**International Prognostic Scoring System**
IPSSWM	**International Prognostic Scoring System for WM**
ISCT	**International society of cellular therapy**
ISS	**International staging system**
JACIE	**Joint Accreditation Committee – ISCT & EBMT**
KM	**Kaplan Meier**
LCMM	**Light chain multiple myeloma**
LPD	**Lymphoproliferative disorder**
MA(C)	**Myeloablative (conditioning)**
MCL	**Mantle cell lymphoma**
MDS	**Myelodysplastic syndrome**
MEL100	**Melphalan 100mg/m^2**
MEL140	**Melphalan 140mg/m^2**
MEL200	**Melphalan 200mg/m^2**
MM	**Multiple myeloma**
MRD	**Minimal residual disease**
NHL	**Non-hodgkin lymphoma**
NK cell	**Natural killer cell**
NPM1	**Nucleophosmin**
NRM	**Non-relapse mortality**
OS	**Overall survival**
PBSC	**Peripheral blood stem cell**
PCR	**Polymerase chain reaction**
PFS	**Progression free survival**
PFS2	**PFS on next-line therapy**
PR	**Partial response**
QoL	**Quality of life**
PTCL	**Peripheral T cell lymphoma**
RIC-SCT	**Reduced intensity conditioned stem cell transplant**
SCT	**Stem cell transplant**
SD	**Stable disease**
TAC	**Transplant Accreditation Committee**
TBI	**Total body irradiation**
TCD	**T cell depleted**
TKI	**Tyrosine kinase inhibitor**
TLCO	**Transfer factor of the lung for carbon monoxide**

TRM	**Treatment related mortality**
TTP	**Time to progression**
VGPR	**Very good partial response**
VUD	**Volunteer unrelated donor**
WM	**Waldenströms Macroglobulinaemia**
WPSS	**WHO classification-based Prognostic Scoring System**

CHAPTER ONE

Introduction

1.1 The history of bone marrow transplantation

In 2007, human bone marrow transplantation celebrated its 50th anniversary after fraught beginnings and a multitude of early failures left many to believe it would never succeed.

Animal studies in the 1950s and 1960s lay the essential groundwork for human bone marrow transplantation. Several important concepts were established; that survival could be achieved following a lethal dose of radiation or high dose chemotherapy if followed by an infusion of marrow cells from an animal donor,[1-7] that this could result in ‘secondary syndromes’, what would later be identified as graft-vs-host disease (GVHD)[8] and that this could be ameliorated by giving infusions from DLA (dog leucocyte antigen) littermate matched canines[9-12] and the administration of methotrexate after the infusion.[13-15]

Concurrently, similar observations were seen in humans. The first human allogeneic transplant was reported by Thomas in 1957 but, despite two patients undergoing transient engraftments, all six reported patients died.[16] This and other early attempts at allogeneic transplantation in humans were unsuccessful, most likely due to a combination of poor HLA (human leucocyte antigen) matching, inadequate immunosuppression to allow acceptance of the foreign graft and the complications of graft vs host disease.[17, 18]

As improvements occurred in the understanding of the HLA system, antibiotic and transfusion therapies, further attempts at human allogeneic transplantation began to demonstrate some success. The first successful allogeneic transplant was reported in 1965 in a patient with refractory lymphoblastic leukaemia who received a bone marrow infusion made up in equal parts of six relatives following high dose TBI (total body irradiation). The patient engrafted rapidly and despite developing acute GVHD was in remission 12 months later with erythroid antigen analysis demonstrating they had the phenotype of one male donor.[19] During the course of the 1970s, the use of sibling donors in patients with advanced leukaemia and aplastic anaemia resulted in a small cohort of long term survivors and the concept that allogeneic transplantation could represent a curative treatment strategy[20, 21]

Following the observation that a small cohort of patients with refractory leukaemia could be cured with allogeneic transplantation, this drove haematopoietic stem cell transplantation (HSCT) to be used earlier in the course of the disease and in patients who were in remission with significantly improved survival outcomes.[22-24] Over the course of the 1980s, the successes that had been reported in patients with acute leukaemias led to the utilisation of allogeneic transplantation in other haematological malignancies and congenital haematological disorders such as thalassaemia and sickle cell disease. [25, 26] By the end of the decade, it was also being used for solid tumours.[27]

Despite these successes, at least half of patients were dying of GVHD. The combined use of ciclosporin and short course methotrexate was demonstrated to significantly reduce the occurrence of GVHD and remains standard practice today.[28, 29]

In 1971, the first allogeneic transplant from an HLA-matched unrelated donor was reported.[30] This success led to the foundation of the Anthony Nolan Trust in the UK in 1974 and the use of unrelated allogeneic transplantation grew over the latter course of that decade. Similar registries were formed in other countries and in 1988 Bone Marrow Donors Worldwide was established allowing significant international expansion of unrelated allogeneic transplantation.

In 1983, Santos et al demonstrated that a non TBI-containing conditioning regimen (busulphan and cyclophosphamide) could be used, opening up the use of transplantation to centres that did not have facilities to deliver TBI.[31]

In the late 1970s, the observation of a graft-vs-leukaemia (GvL) effect was reported, firstly following the concurrent achievement of remission following a graft-vs-host reaction and subsequently the demonstration that the occurrence of GVHD was associated with a reduction in disease relapse.[32, 33] Over a decade later, further demonstration of the GvL effect was reported with the use of immunosuppression withdrawal and the use of donor lymphocyte infusions (DLI) to achieve remission in those who relapsed post transplant.[34, 35] The understanding that tumour cells could be eliminated by donor T cells rather than high doses of radiotherapy and chemotherapy led to the development of reduced intensity conditioning (RIC). [36-38] Harnessing the immunological power of allogeneic transplantation and consequent DLI infusions to manipulate disease control opened up the doors of HSCT to older and less fit patients. Furthermore, the significant morbidity and mortality associated with myeloablative transplantation was significantly less using this approach. T cell depletion (TCD) was introduced as a strategy to try and avoid the complications of T cell mediated GVHD. However, it was associated with increased graft failure and relapse rates attributable to the loss of the graft-vs-tumour effect in addition to increased viral complications.[39] This increased relapse risk was reported to be offset by repeated DLI infusions and TCD remains a favoured conditioning strategy in some centres, particularly where the risk of GVHD is higher, e.g. where there is an unrelated or mismatched donor.

The headway made in allogeneic transplantation re-ignited enthusiasm for autologous stem cell transplantation (ASCT) in the 1980s. Avoiding the need to identify an HLA-matched donor and the risks of graft versus host disease made this an attractive strategy. Early reports demonstrated significant morbidity and mortality but there were some successes in patients with leukaemia, myeloma and lymphoma.[40-43]

In the late 1980s, the use of peripheral blood stem cells (PBSCs) rather than bone marrow began to be used in humans after its success had been demonstrated in animal studies. The use of chemotherapy and haematopoietic growth factors resulted in increased circulating PBSCs[44, 45] and stem cells could be separated based on their CD34 expression.[46] Although the use of PBSCs carried an increased risk of GVHD when used in allogeneic transplantation, the higher number of stem cells compared to bone marrow harvests resulted in more rapid engraftments. Progress in cryopreserving autologous PBSC when patients were in remission was a significant step forwards in autologous transplantation. However, the difficulties of transplanting a graft that was not entirely disease free resulted in early relapses and despite attempts to purge the stem cell grafts, this problem has not been overcome.[47-49] Despite this, ASCT continues to offer long remissions and improved overall survival and today accounts for the majority of stem cell transplant procedures.[50, 51]

Following the demonstration that haematopoietic stem cells were present in cord blood, in 1989 the first successful cord stem cell transplant (SCT) was reported.[52] Following this, cord banks have been established in many centres offering potential stem cell donor options to those without sibling or matched unrelated donors.

1.2 Where are we today?

Over half a century since the first human allogeneic transplant, despite many obstacles and significant early failures, E. Donnall Thomas spearheaded progress in the field of stem cell transplantation (and was awarded the Nobel Prize for his pioneering efforts in 1990). Today, successful stem cell transplants are carried out for a variety of haematological and non-haematological malignancies, congenital haematological disorders, autoimmune conditions and inherited metabolic disorders. The formation of donor registries and cord banks in combination with advances in HLA typing to include molecular rather than serological typing mean that suitable stem cell donors are not only matched siblings, but also include matched volunteer unrelated donors (VUD), related haploidentical donors and cords with over 23 million donors available worldwide.[53] Improvements in supportive care have occurred with the development of hickman lines, better antibiotic, antiviral and antifungal therapies, pre-emptive CMV DNA PCR analysis and treatment, improved blood product support and treatment of GVHD have all contributed to improved outcomes over the last few decades. Furthermore, clinicians now have validated tools to help identify which patients are fit to proceed with transplantation.[54] The 1980s saw the introduction of transplant registries such as the European Group for Blood and Marrow Transplantation (EBMT) and the Centre for International Blood and Marrow Transplant Research (CIBMTR) playing a bigger role in HSCT with working parties providing education, standards of practice and a means of collecting and analysing outcome data.

There has been remarkable progress but there is a long way to go. The morbidity and mortality associated with allogeneic transplantation remains significant. Autologous transplantation is a safer procedure but does not offer the same curative potential. Consequently, decisions about which patients should be transplanted, how and when, continue to cause controversy. The introduction of new drugs are muddying the water further as their improved clinical responses and survival outcomes are now calling into question whether transplantation will continue to have a role at all in several disease settings.

1.3 The British Society of Blood and Marrow Transplantation

Over the last two decades, there has been a significant rise in the number of SCTs being performed in the UK. During this period of growth, it became apparent that in order to ensure stem cell transplantation was being performed to the highest standards, monitoring was required. The British Society of Blood and Marrow Transplantation (BSBMT) was formed in 1995. At inception, its objectives were to define the role and to monitor the outcomes of patients having stem cell transplant procedures in the United Kingdom and Ireland. Consequently, it became responsible for collecting data on all stem cell transplants performed within all 55 BSBMT member centres in the UK and Republic of Ireland. In addition to these initial aims, the BSBMT has extended its role as listed below.

1.3.1 Stem cell transplant data collection

As already mentioned, collating and maintaining a robust and complete data-set of all UK stem cell transplants lies at the heart of BSBMT. The EBMT produces data collection forms entitled MED-A and MED-B. The MED-A comprises two forms which are considered the Minimum Essential Data and the completion and submission of these forms is mandatory in order for a centre to hold full EBMT membership. The first report enables patient registration. This form collects data on patient age, gender and ethnicity, disease, performance score, CMV status, serology status and history of fungal infection pre-transplant. Information is also collected regarding SCT type, stem cell source, whether there has been any graft manipulation ex-vivo in addition to route of cell infusion, cell count and viability. HLA-match type, donor gender, donor CMV/serology status, chronological number of transplant type, conditioning regimen and whether myeloablative or not is also required information for submission. Post-transplant data on type of GVHD prophylaxis used, engraftment data, chimerism data, the occurrence and treatment of acute GVHD or other complications within the first 100 days and whether further cellular products have been given is collected in addition to disease status at day 100. There is also an annual follow-up report that records post transplant complications. BSBMT is responsible for ensuring that annual follow-up data is submitted. It is also their role to check data quality and to clarify and request missing data.

The MED-B forms consist of disease specific and HSCT specific forms (allograft and autograft). They collate detailed pre-transplant information on diagnosis, subtypes, cytogenetics etc, prior treatment and response in addition to detailed recording of post transplant complications. The MED-B forms are not yet mandatory but form the basis of registry-based scientific studies and all transplant centres are encouraged to submit them in order to strengthen the data that can be produced.

BSBMT produces an annual report with analysis of a rolling 5 year cohort in addition to a more comprehensive analysis of the previous year's activity. Analysis includes patient demographics, logistical data (graft source, time to transplant), non-relapse mortality (NRM) at D100 and 1 year and overall survival (OS) at 1, 2 and 5 years in addition to transplant-related complications and late effects. There are sub-analyses both by indication for transplantation and by transplant type. Transplant activity is compiled both by centre and region. The most recent was published in 2014 in relation to UK transplant activity between 2006 and 2011.

1.3.2 Transplant centre accreditation

In 1998, the EBMT and the International Society of Cellular Therapy (ISCT) established the Joint Accreditation Committee – ISCT & EBMT (JACIE). Its role is to provide an inspection-based assessment and accreditation process against established standards in stem cell transplantation, promoting high quality patient care and laboratory performance. JACIE is a committee of the EBMT and data submission to EBMT is required in order to achieve successful accreditation with JACIE. The BSBMT acts as the interface between the EBMT and all UK transplant centres. It is the responsibility of BSBMT to ensure all UK transplant data is submitted to EBMT. The Transplant Accreditation Committee (TAC), a BSBMT sub-committee, holds the duty of ensuring that sufficient laboratory, clinical and stem cell collection facility inspectors are trained for the purposes of JACIE inspections and accreditation. It is also responsible for organising the inspections themselves. The BSBMT

JACIE inspectors will provide transplant centres with detailed reports, following review by the JACIE office and medical director.

1.3.3 Benchmarking

All collected outcome data is collated so that benchmarking can occur. Local activity and outcome data is compared to the collated national data. This information is sent back to each UK transplant centre on an annual basis so that individual centre performance can be evaluated and compared to a unified national outcome standard. In addition to this, BSBMT also makes a comparison of UK data to the annual EBMT Transplant activity to allow international benchmarking to take place.

1.3.4 Supporting clinical transplant decision making and transplant commissioning

The BSBMT committee have produced a table which reviews the indications for transplant by disease type. Its aim is to provide an up-to-date, evidence-based guidance for which clinical scenarios stem cell transplantation should be performed in. It is recommended that this table is referred to by all transplant physicians and purchasers. There are separate tables for adult and paediatric practice. This data is used to support the Clinical Reference Group that provides guidance and strategic direction for the commissioning of stem cell transplantation.

The table considers individual diseases by subtype and response to treatment. It then considers the role of autologous transplantation, sibling or unrelated donor allogeneic transplantation in each scenario. Recommendations are categorised into óSô which is the standard of care, óCOô which indicates the transplant is a clinical option and may be considered after assessment of risks and benefits. óDô indicates that performing a transplant in such a scenario is ódevelopmentalô and further trials are required before this could be recommended and finally óGNRô suggests a transplant would be generally not recommended. The BSBMT committee members that formulated this table also serve as an adjudication committee in scenarios where there are funding disputes or less common situations which may not be listed in the table. The committee serve to provide impartial expert advice about whether the transplants in discussion are appropriate.

1.3.5 Training and education

The BSBMT has a responsibility to provide training and support for UK transplant data managers. In addition to this it provides documents, guidelines and training days in relation to stem cell transplantation practice.

1.3.6 Clinical trials

The óclinical trials committeeô is a BSBMT sub-committee, involved in overseeing both retrospective and prospective stem cell transplant studies with the aim of informing and directing UK stem cell transplantation.

1.3.7 BSBMT scientific sub-committee

Through the organisation of meetings and workshops, this subcommittee aims to encourage and develop the interactions between clinicians and scientists working in stem cell transplantation, in order to facilitate improved clinical-scientific research projects and collaborations.

1.4 Summary of UK stem cell transplant outcome data

In 2014, the BSBMT 5th report to specialist commissioners was published with a summary of outcome data for UK stem cell transplants performed between 2006 and 2011 in addition to a detailed analysis of transplant activity and outcomes in 2012.[55] Between 2006 and 2011, 15,088 adult transplants were carried out in the UK providing further evidence that transplant activity in the UK is rising year on year. The majority were first transplants with second and subsequent transplants accounting for just over 10%.

Over 60% of all SCT procedures were autologous stem cell transplants and just over 50% of these were for patients with multiple myeloma. The BSBMT report confirms that this is a relatively safe procedure with an overall NRM of 2% and 4% at 100 days and 1 year respectively. Five year overall survival (OS) is 60%. Data for those aged over 60 years is comparable but they have a slightly higher NRM and consequently a lower 5 year OS.

Analysis of allogeneic transplants revealed demonstration of the increasing reliance on non-sibling donors. There were 2570 VUD transplants compared to 1903 sibling transplants. 206 transplants were from alternative donors. The stem cell source was PBSC in 90%. 3% of adult transplants utilised cord blood cells. The use of RIC far exceeded myeloablative (MA) conditioning for almost all disease types with no difference in NRM and 1 year OS between the two conditioning approaches. Exceptions to this were in acute lymphoblastic leukaemia (ALL) where more ablative transplants are still being performed. The report comments that this disease is more common in the younger population and the efficacy of RIC transplantation is reported to be less clear compared to many other haematological malignancies, therefore explaining this discrepancy. There were also more ablative transplants performed in chronic myeloid leukaemia (CML) and myeloma, most likely attributable to patient selection given that allogeneic transplantation for both disease subtypes is not standard of care.

NRM at 100 days and 1 year was reported as 8% and 16% for siblings and 11% and 24% for VUD transplants respectively. Overall 5 year survival for siblings was 53% with 41% for VUDs. As with the autologous recipients, those over 60 years were identified to have marginally worse 5 year survival outcomes.

Grade 3 or 4 acute GVHD was reported to affect 6% and 7% of sibling and VUD transplants respectively. 46% of adult patients were reported to develop chronic GVHD although with only 5% of all allogeneic transplant recipients developing extensive chronic GVHD.

1.5 Summary of St Bartholomew's outcome data from the BSBMT

Between 2006 and 2011, 638 transplants were carried out at St Bartholomew's Hospital; 544 were first transplants which form the basis of the BSBMT centre analysis. Of these, 361 were autologous SCTs, 87 were sibling allogeneic transplants, 93 were VUDs and 3 were from alternative donors (1 cord, 1 syngeneic and 1 mismatched family donor).

Table 1 summarises the outcomes of autologous transplants at St Bartholomew's in comparison to the BSBMT data over the same time period. Overall survival at 1 year and 5 years is 88% and 53% respectively for the autologous SCT cohort. NRM at D100 and 1 year is 4%, comparable to BSBMT data. 202 ASCTs were for patients with multiple myeloma, 37 for Hodgkin lymphoma and 77 for non-hodgkin lymphoma. Overall survival for all three disease subtypes was comparable or even superior to BSBMT data up to 2 years post transplant. However there is then a tailing off with inferior 5 year OS for multiple myeloma. No absolute numbers are provided in the BSBMT centre specific report but the St Bartholomew's confidence intervals are much wider than the UK BSBMT dataset, suggesting that these discrepancies should be treated with caution. Similarly there is a discrepancy in 2 and 5 year NRM outcomes across all disease types. The rising NRM over time is odd in the context of ASCT, as deaths beyond this time point are almost always disease related. Furthermore, this BSBMT data is discrepant with local data where NRM beyond 2 years is static. This leads either the accuracy of BSBMT data submission or methods for analysis to be questioned. Patients are often treated at more than one centre, and towards the end of life the majority of their care may occur locally rather than at their transplant centre. Consequently, clinical details at this stage are not always available or clearly documented. I suspect that some of the patients who are currently falling into the bracket of NRM have

Table 1: Autologous stem cell transplant outcomes between 2006 and 2011

(Amalgamation of BSBMT 5th report to specialist commissioners and St Bartholomewӳ Centre report)

	All ASCT Barts	All ASCT BSBMT	MM Barts	MM BSBSMT	HL Barts	HL BSBMT	NHL Barts	NHL BSBMT
Number	361 (316 MM/HL/NHL)*	8552	202	4578	37	918	77	2535
% engraftment failure	1	1	0	0	0	1	4	1
Med engraftment time (d)	15	12	15	13	14	11	18	11
% OS 1 year (95% CI)	88 (84-91)	89(88-89)	93 (89-96)	93(92-93)	95(80-99)	92(90-93)	88(79-94)	83(81-84)
%OS 2 years (95% CI)	80(75-83)	80(79-81)	85(79-89)	84(83-86)	89(74-96)	84(82-87)	77(27-60)	74(72-76)
%OS 5 years (95% CI)	53(46-59)	60(59-62)	49(39-58)	59 (57-61)	76(55-88)	69(65-73)	57(42-70)	60(58-63)
NRM D100 (95% CI)	4 (2-6)	2(2-3)	2 (1-5)	1 (1-2)	5(1-16)	3(2-4)	8(3-15)	4(3-5)
NRM 1 year (95% CI)	4(3-7)	4(4-5)	3 (1-6)	3(2-3)	5(1-16)	4(3-6)	8(3-15)	6(5-7)
NRM 2 years (95% CI)	7(4-10)	6(5-6)	5(2-8)	4(3-5)	11 (3-23)	7(5-8)	15(8-23)	8(7-9)
NRM 5 years (95% CI)	12 (8-17)	10(10-11)	18(12-26)	8(7-9)	16 (5-31)	12(9-14)	32 (19-46)	14(13-16)
% secondary malignancy	1	1	2	2	0	2	2	3
% late graft loss	0	0	0	0	0	0	0	2
% conception post SCT	1	0	0	0	7	3	0	0

ASCT autologous stem cell transplant; Barts St Bartholomewӳ Hospital; BSBMT British society of Blood and Marrow Transplantation; OS overall survival; NRM non-relapse mortality; MM multiple myeloma; HL Hodgkin Lymphoma; NHL Non-hodgkin lymphoma

*39 done for solid tumours and 6 ӭiscellaneousӠ data for these sub-groups not included in this table actually relapsed and died of relapsed disease but that these events have not been captured.

Tables 2, 3 and 4 summarise the outcomes of allogeneic transplants performed at St Bartholomew's between 2006 and 2011, again in comparison to collated BSBMT UK data. Over 80% of both sibling and VUD transplants utilised RIC platforms. This is more than the overall UK data, which reports 61% of sibling and 69% of VUD transplants were reduced intensity. Without more data on factors that influence conditioning selection such as age and comorbidities, it is difficult to analyse this discrepancy further. It is interesting to note that no identified differences in survival outcome were seen in the BSBMT comparison between the two conditioning strategies.

Looking at data for all allogeneic transplants, NRM data both for siblings and VUDs is higher at all time-points compared to BSBMT data, with a resultant negative impact on OS data. Importantly, the Kaplan Meier curves provided with the centre report demonstrate that outcomes for allogeneic transplantation lie within the BSBMT outcome confidence intervals.

Making sense of these inferior percentages is difficult. Firstly there is a large patient number discrepancy between the St Bartholomew's and UK data. Relatively small numbers of patients and consequent events e.g. death will have a relatively large impact on percentages in terms of OS and NRM. Furthermore, even when the datasets are broken down into disease cohorts (tables 3 & 4), it is unclear whether these datasets are directly comparable. Across all UK transplant centres, there will undoubtedly be differences in clinical practices. Different first line treatments, salvage therapies and transplant conditioning regimens will be used. Selection criteria for transplant or type of transplant in terms of age and comorbidities may differ. Patient risk assessments in terms of their need for transplant may again vary across different centres. All of these factors will come into play when analysing outcomes and in particular when making comparisons.

If the outcomes are considered by individual disease-types (where data is available), the numbers suggest that outcomes for those with AML in CR1 and with Hodgkin lymphoma are relatively favourable compared to the UK BSBMT data. Those with AML not in CR1 and CLL appear to do worse. As already mentioned, with the even lower patient numbers once divided by diagnosis and wide confidence intervals, it is difficult to draw conclusions.

Table 2: Allogeneic stem cell transplant outcomes between 2006 and 2011

(Amalgamation of BSBMT 5th report to specialist commissioners and St Bartholomew's centre report)

	All SIB Barts	All SIB BSBMT	All VUD Barts	All VUD BSBMT
Number	87	1903	93	2570
% RIC	83	61	87	69
% engraftment failure	2	2	2	3
Med engraftment time (d)	17	14	17	13
% OS 1 year (95% CI)	64(53-73)	71(69-73)	54(43-64)	62(60-64)
%OS 2 years (95% CI)	57(46-67)	63(60-65)	46(36-56)	51(49-53)
%OS 5 years (95% CI)	45(34-55)	53(51-56)	32(21-43)	41(39-44)
NRM D100 (95% CI)	11 (6-19)	8(7-9)	22 (14-31)	11(10-12)
NRM 1 year (95% CI)	22(14-31)	16(14-18)	29(20-39)	24(22-26)
NRM 2 years (95% CI)	23(15-32)	19(17-21)	35(25-45)	30(28-32)
NRM 5 years (95% CI)	27(18-37)	23(21-25)	45(33-56)	35(33-38)
%Acute GVHD	11	34	9	49
% Grade 3/4 aGVHD	6	6	6	7
% Chronic GVHD	40	NR	40	33
% Extensive cGVHD	3	NR	3	5
% secondary malignancy	2	2	2	2
% late graft loss	0	1	0	1
% conception post SCT	1	1	0	0

SIB Sibling; VUD Volunteer unrelated donor; NR Not reported

Table 3: Allogeneic stem cell transplant outcomes for leukaemia between 2006 and 2011
(Amalgamation of BSBMT 5th report to specialist commissioners and St Bartholomew's Centre report)

	AML in CR1		AML not in CR1		ALL in CR1		ALL not in CR1		CML in CP1		CML not in CP1		CLL		Other Leukaemias	
	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT
Number	26	865	33	691	13	426	6	128	3	147	4	123	20	278	11	191
% RIC	85	67	88	61	31	25	50	18	67	48	75	40	100	86	82	58
% engraftment failure	0	2	3	2	0	3	0	5	0	3	0	6	5 (n=1)	4	0	5
Med engraftment time (d)	16.5	13	17	13	17	14	23.5	15	16	15	15.5	15	16	14	17	14
% OS 1 year (95% CI)	76(55-89)	71(68-74)	47(29-63)	53(49-57)	62(31-82)	66(61-71)	NR	47(38-56)	NR	NR	NR	66(56-71)	65(40-82)	71(65-76)	82(45-95)	59(52-66)
%OS 2 years (95% CI)	72(51(86)	60(56-63)	34(19-51)	41(37-45)	62(31-82)	58(53-63)	NR	41(32-50)	NR	NR	NR	58(47-66)	60(36-78)	62(55-67)	64(30-85)	46(38-53)
%OS 5 years (95% CI)	63(40-79)	47(43-51)	18(7-35)	33(29-38)	NR	49(43-54)	NR	30(20-39)	NR	NR	NR	45(35-55)	44(22-64)	51(44-58)	55(23-78)	39(30-47)
NRM D100 (95% CI)	NR	5(4-7)	16(6-30)	11(9-13)	15(2-39)	13(10-16)	NR	16(10-22)	NR	5(3-10)	NR	12(7-19)	25(9-45)	11(8-15)	9(1-33)	12(8-17)
NRM 1 year (95% CI)	NR	15(12-17)	25(12-41)	23(20-26)	23(6-47)	24(20-29)	NR	26(18-34)	NR	15(10-22)	NR	22(15-20)	35(16-55)	25(20-31)	9(1-33)	24(18-31)
NRM 2 year (95% CI)	NR	19(16-22)	31(16-47)	28(24-31)	23(6-47)	27(23-32)	NR	29(21-37)	NR	19(13-26)	NR	24(16-32)	35(16-55)	30(24-36)	9(1-33)	30(24-37)
NRM 5 years (95% CI)	5(0-20)	24(21-28)	40(23-57)	31(27-35)	23(6-47)	32(27-37)	NR	33(24-42)	NR	27(19-36)	NR	29(20-38)	40(19-61)	36(30-43)	18(3-44)	31(24-38)
%Acute GVHD	12	42	26	46	15	50	50	60	33 (n=1)	41	25 (n=1)	47	30	46	0	47
% Grade 3/4 a GVHD	4	5	10	7	0	9	17	11	33	10	0	8	5 (n=1)	12	0	11
% Chronic GVHD	48	44	47	49	67	52	20 (n=1)	46	100 (n=1)	45	50 (n=1)	42	79	56	50	50
% Extensive cGVHD	0	7	5	7	11	8	20	8	100	9	0	7	0	12	0	7
% secondary malignancy	5(n=1)	2	0	3	0	0	0	0	NR	2	NR	0	14 (n=2)	6	0	3
% late graft loss	0	0	0	1	0	1	0	1	NR	2	NR	1	0	2	0	2
% conception post SCT	5(n=1)	1	0	0	0	2	0	0	NR	0	NR	0	0	1	0	0

CR1 Complete remission 1; **CP1** Chronic Phase 1; **NR** Not reported

Table 4: Allogeneic stem cell transplant outcomes for non-leukaemia between 2006 and 2011

(Amalgamation of BSBMT 5th report to specialist commissioners and St Bartholomew's Centre report)

	MM		HL		NHL		MDS		BM aplasias		MPS	
	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT	Barts	BSBMT
Number	2	54	1	119	30	570	22	703	6	185	6	197
% RIC	50	31	100	72	100	82	100	85	50	62	100	83
% engraftment failure	0	4	0	2	0	3	9(n=2)	4	0	9	0	4
Med engraftment time (d)	13	15	11	12	16	13	20	13	17.5	17	17	16
% OS 1 year (95% CI)	NR	68(53-79)	NR	77(68-84)	77(57-88)	65(61-69)	41(21-60)	62(58-66)	NR	83(77-88)	NR	66(58-72)
%OS 2 years (95% CI)	NR	63(48-75)	NR	72(62-79)	67(47-80)	58(54-62)	41 (21-60)	49(45-53)	NR	81(74-86)	NR	55(47-62)
%OS 5 years (95% CI)	NR	47(31-62)	NR	61 (47-72)	54(32-73)	50(45-55)	41 (21-60)	41(37-45)	NR	81(74-86)	NR	38(28-48)
NRM D100 (95% CI)	NR	15(7-26)	NR	12(7-18)	20(8-36)	11(9-14)	14(3-31)	11(9-14)	NR	10(6-15)	NR	9(5-13)
NRM 1 year (95% CI)	NR	25(14-38)	NR	16(10-23)	23(10-39)	24(20-27)	32 914-51)	24(20-27)	NR	15(10-20)	NR	23(17-29)
NRM 2year (95% CI)	NR	25(14-28)	NR	19(12-27)	33(18-50)	28(24-32)	32(14-51)	31(27-34)	NR	17(12-23)	NR	30(23-37)
NRM 5 years (95% CI)	NR	35(21-50)	NR	27(16-39)	46(24-65)	33(28-37)	32 (14-51)	35(31-38)	NR	17(12-23)	NR	36(28-44)
%Acute GVHD	0	55	100	38	28	43	14	42	33	18	20	41
% Grade 3/4 a GVHD	0	14	0	4	10	6	5	7	0	1	20	12
% Chronic GVHD	50 (n=1)	42	NR	39	82	47	50	44	50 (n=1)	20	80	46
% Extensive cGVHD	0	6	NR	1	5	6	0	7	50(n=1)	2	0	5
% secondary malignancy	NR	0	NR	0	0	2	NR	5	NR	4	20 (n=1)	2
% late graft loss	NR	0	NR	1	0	2	NR	2	NR	3	0	3
% conception post SCT	NR	6	NR	7	0	0	NR	1	NR	4	0	0

NR Not reported MPS Myeloproliferative syndromes

There is a striking difference in the incidence of acute GVHD reported with less than 10% at St Bartholomew's compared to 34% and 49% for siblings and VUDs respectively across the UK. Local analysis quantifies the incidence of chronic GVHD at approximately 60%. This is in the setting of RIC-SCT which accounts for the majority of transplant procedures. This conditioning platform results in a small percentage of historically defined acute GVHD occurring before 100 days, consistent with the figures of 9-11% seen in table 2. However much of the 'chronic' GVHD that occurs is actually late onset acute GVHD that occurs on tailing of ciclosporin. For the majority of patients, immunosuppression withdrawal commences at day 90 and therefore these events are falling into the historically defined chronic GVHD bracket, hence the low reported incidence of acute GVHD and the higher incidence of chronic GVHD. Much of this is not limited or extensive chronic GVHD but rather grades II and III acute GVHD, with a small proportion of grade IV.

The reclassification of GVHD and perhaps unclear documentation of grades of GVHD means that data reporting by non-clinical data managers is not likely to be entirely representative of true clinical practice.

1.6 Limitations of the British Society of Blood and Marrow Transplantation

The BSBMT centre report provides centre–specific Kaplan-Meier OS curves which demonstrate that St Bartholomew's outcomes across all transplant types fall within the confidence intervals of the UK BSBMT data. To glean any other valid conclusions about local practice in comparison to the UK from the data provided in the BSBMT reports is difficult and limits the utility of this benchmarking exercise. Clearly collating data for over 15,000 transplants from over 50 different centres is not an easy undertaking and a balance must be struck of what data can be obtained to provide a robust, informative data-set in a timely manner. Not all detailed data collected from the MED-A and MED-B forms is provided in the BSBMT reports. To produce an annual report with details regarding a patient's prior treatments, performance score, comorbidities, conditioning regimen, to name but a few, would be a much more difficult undertaking and perhaps this is why only the most salient outcome data is provided, reserving the details for more comprehensive retrospective studies. This means that the annual report serves to ensure that centres are on the right track, but does not provide information that could serve to modify clinical practice.

The BSBMT indications table aims to provide recommendations that are evidence-based where possible with references to support these recommendations. However, the guidance is broad. For example, in the guidance for myeloma, it is recommended that an autologous stem cell transplant is the first-line standard-of-care óin patients suitable for intensive treatmentô but does not define what makes a patient suitable.

It recommends a sibling allogeneic transplant in the first-line treatment of myeloma and states that suitability for a myeloablative versus reduced-intensity approach should be based upon biological suitability which includes assessment of age, comorbidities, advanced disease stage etc, without giving any strict definitions or guidance. A matched-unrelated-donor allogeneic transplant is a clinical option in óselected patientsô both first-line and at relapse but does not define which patients fit the óselectedô criteria. There are many scenarios in the table where transplants are a óclinical optionõ, usually reflecting a lack of consensus in the literature about the role of SCT in those scenarios.

This broad guidance is necessary when creating a document that so many individual clinicians and transplant centres are required to follow. It would be near impossible to create guidelines with more strict definitions that all transplant centres would adhere to. Assessment of whether a patient is suitable for intensive treatment can cause disagreement between two clinicians so to provide detailed guidance with an expectation that all treating centres would adhere to it would be an unrealistic goal. Ultimately, these treatment decisions are made at the discretion of the treating clinician overseen by the multi-disciplinary team and local guidelines in that centre.

The result of this, is that whilst all centres are following the same BSBMT guidelines, there may still be significant differences in which patients are receiving which types of stem cell transplants. This is particularly the case in scenarios where definitions are vague and the evidence is weak, such as age cut-offs or assessments of performance status.

Lower-dose conditioning in autologous transplantation and RIC in allogeneic transplantation has resulted in patients with a lower performance status and of an older age to become eligible candidates for transplantation procedures.[36, 37, 56] Whilst this has allowed SCT to become a viable treatment option for a wider range of patients, it also means there is an increasing grey area over which patients are appropriate for transplant.

I have identified four clinical scenarios/areas where the BSBMT guidelines are not clear about the specifics of practice but in which we have specific clinical experience at St Bartholomewõs. I aim to review outcomes with the aim of trying to identify if more specific recommendations can be made within those scenarios which continue to cause controversy.

I will discuss the background, methods, results and discussion of each separately in this report. They are:

1) Autologous stem cell transplantation in multiple myeloma ï comparing outcomes of two melphalan conditioning doses

2) Allogeneic stem cell transplantation in multiple myeloma

3) Sequential transplantation in refractory and relapsed acute myeloid leukaemia and myelodysplasia

4) Allogeneic stem cell transplantation in lymphoma

CHAPTER TWO

Autologous Stem Cell Transplantation in Multiple Myeloma

2.1 Introduction

Autologous stem cell transplantation in first response is considered the standard of care for younger patients with newly diagnosed Multiple Myeloma (MM) with increased progression-free survival (PFS) and OS rates compared to conventional chemotherapy [50, 57, 58].

Melphalan 200mg/m^2 (MEL200) is considered the standard conditioning regimen for patients aged less than 65 years.[59] Concerns over increased toxicity and treatment related mortality (TRM) have generally restricted the use of this conditioning regimen in older patients. However, the median age of diagnosis in patients with MM is approximately 70 years with only 15% of patients reported to be less than 60 years at diagnosis. [60]

Dose reduction of melphalan conditioning is well recognised in those with renal impairment.[61] However, within the limited published data on how best to condition older patients, or those with comorbidities, there is little consensus on the optimal conditioning regimen that should be used.

Mixed results have been reported regarding the use of MEL200 in older patients. Equivalent survival has been reported using MEL200 conditioning when comparing patients aged >65 years to younger, matched patients.[62-65] However, there is likely to be considerable patient selection behind these equivalent outcomes. Only ‘older’ patients who were deemed fit for this approach by their treating physicians will have undergone this approach. Others have reported that whilst MEL200 provides a survival advantage compared to conventional chemotherapy in those aged >60 years, this is inferior to the survival advantage achieved by patients <60 years. [66]

Badros et al reported a TRM of 16% in patients >70 years receiving MEL200.[56] As a result, they reduced their melphalan conditioning dose to 140mg/m^2 (MEL140) for subsequent patients in this age group, and observed a reduction in TRM to 2%. Equivalent PFS and OS rates were achieved irrespective of dose. 44% of these patients went on to have a second ASCT at doses of either 200mg/m^2 or 140mg/m^2, making it difficult to evaluate the overall effect of dose on OS and PFS in the longer-term.

Following on from this study, rather than not transplant at all, some centres elected to give older patients reduced-dose conditioning in whom there were concerns about fitness to tolerate MEL200. More recently, studies have reported on the safety and feasibility of ASCT in patients over the age of 65, with the majority of patients receiving MEL200, but a subgroup in each study receiving varying doses between 100mg/m^2, 140mg/m^2 or 180mg/m^2. Little information is provided regarding the rationale for dose reduction and outcomes are based on the population as a whole, with no comment regarding the relative outcomes of the differing conditioning approaches.[67-69]

Studies have reported mixed results using melphalan 100mg/m^2 (MEL100). Whilst it has been reported that MEL100 is superior to conventional chemotherapy,[70] this has not been confirmed in other studies looking specifically at patients aged >65.[71] In studies comparing MEL100 to MEL200 in patients under the age of 65, although there was a significantly increased PFS with MEL200, this did not translate into a survival advantage.[72]

When MEL140 plus 8Gy TBI was compared to MEL200, PFS and OS were found to be equivalent but the former was associated with higher toxicity. Thus, it was concluded that MEL200 was less toxic and should be the standard of care.[73]

BSBMT guidelines recommend a first ASCT in myeloma patients 'suitable for intensive treatment'. There are however no specific guidelines about what determines suitability and certainly no age thresholds, comments on performance status or indeed the type or dose of conditioning (table 5).

Table 5: BSBMT indications for SCT in myeloma (taken from BSBMT Indications for SCT version Oct 13)

Myeloma

	Sibling transplant‡	**MUD transplant**	**First Autograft**	**Second Autograft**
First Line	S[16, 17]	CO[18] -Selected patients or as part of clinical trial	S[9] -for patients suitable for intensive treatment	CO[10,11] (Tandem autograft may be considered if no CR after 1st autograft)
Relapse	CO[12, 19]	CO -Selected patients or as part of clinical trial	S (If not done in first response but patient is considered fit)	S[13] -If time to re-treatment after 1st autograft >18m or as part of NCRN Myeloma X trial
Plasma cell leukaemia	S[15] -If chemo responsive disease -Selected young patients <55 years	CO[15] -If chemo responsive disease	S[15] -If no suitable donor or unfit for allograft	CO

‡ - Suitability for a myeloablative versus reduced-intensity is based on biological suitability (age, co morbidity, advanced disease stage, etc)

Based upon the standard practice of using MEL140 in patients with renal impairment, practice at St Bartholomew's has been extended to give MEL140 to patients aged >65 years and those with comorbidities in order to minimise anticipated excess toxicities in these patient groups.

2.2 Hypothesis

Patients who receive MEL140 have poorer outcome because of comorbidities and the use of lower dose of chemotherapy.

2.3 Methods

2.3.1 Patients' characteristics

A total of 253 patients with multiple myeloma underwent ASCT at St Bartholomew's Hospital between November 2004 and November 2011. Patients were excluded from further analysis if they had received a previous ASCT (n=29), if they went on to consolidate treatment with either maintenance chemotherapy (n=9) or allogeneic stem cell transplantation (n=6), if they received melphalan conditioning at 100mg/m^2 (n=9) or if they received MEL140 on account of poor stem cell harvest (n=2). Five patients were lost to follow-up. The remaining 193 patients are included in this analysis.

2.3.2 Induction therapy, PBSC mobilisation and autografting

Patients received a variety of induction chemotherapy regimens as detailed in table 6. The higher percentage of patients with renal impairment in the MEL140 group explains the higher use of bortezomib-based induction chemotherapy in this category, in line with current treatment recommendations.[61] Where more than one line of therapy was utilised prior to ASCT, this was because there was either progressive disease, stable disease or a sub-optimal response to initial therapy and further therapy was given to maximise response before ASCT.

Patients had stem cells mobilised using cyclophosphamide priming (1.5g/m^2), followed by Granulocyte Colony-Stimulating Factor (G-CSF) administration from day 3.

Patients received MEL140 or MEL200 on Day-2 or MEL140 on Day-3 if the creatinine clearance was <50ml/min. On Day D0, cryopreserved stem cells were re-infused and G-CSF was commenced on Day+4 and continued until neutrophil recovery (>1x10^9/L).

All patients received antimicrobial prophylaxis with aciclovir, fluconazole and ciprofloxacin. All patients were commenced on cotrimoxazole following engraftment as prophylaxis against *Pneumocystis jiroveci*.

Table 6: Myeloma ASCT: Patient characteristics according to treatment group

		MEL200	MEL140	P value
No. of patients		140	53	-
Male sex (%)		88 (62.8)	31 (58.5)	0.711
Age (median)[range]		56 [28-67]	64 [41-72]	<0.001
Myeloma Isotype	**IgG (%)**	87(62.1)	26(49.1)	0.039
	IgA(%)	24(17.1)	7(13.2)	0.362
	Light Chain Myeloma (LC-MM)(%)	22(15.7)	20(37.7)	0.002
	Non-secretory(%)	5(3.6)	0	-
	Not known	2(1.4)	0	-
International Staging Score (ISS) at diagnosis	**1(%)**	54(38.6)	12(22.6)	0.029
	2 (%)	33(30)	4(7.5)	0.005
	3 (%)	23(16.4)	27(58.5)	P<0.001
	Not known (%)	30(21.4)	10(18.9)	-
Creatinine Clearance (ml/min) median		108	79.0	<0.001
Pre ASCT induction	**Bortezomib based(%)***	12(8.6)	7(13.2)	0.425
	Thalidomide based(%)**	88(62.9)	31(58.5)	0.642
	Lenalidomide based (%)$^{+}$	2 (1.4)	0	-
	>1 line of novel-agent(%)	10(7.1)	9(16.9)	0.041
	No novel agent(%)***	28(20)	6(11.3)	0.158
Pre ASCT response status	**CR(%)**	34(24.3)	8(15)	0.199
	VGPR(%)	13(9.3)	11(20.8)	0.020
	PR(%)	80(57.1)	30 (56.6)	0.821
	RR(%)(≥PR)	127 (90.7)	49(92.5)	-
	SD(%)	13(9.3)	4(7.5)	0.704

*VD, velcade & dexamethasone; PAD, velcade, doxorubicin & dexamethasone

**CTD, cyclophosphamide, thalidomide & dexamethasone; TD, thalidomide & dexamethasone

$^{+}$ RCD, lenalidomide, cyclophosphamide & dexamethasone

***VAD, vincristine, doxorubicin & dexamethasone; CVAD, cyclophosphamide, vincristine, doxorubicin & dexamethasone

CR Complete response; VGPR Very good partial response; PR Partial response; RR Response rate; SD Stable disease

2.3.3 Response criteria

Disease response was assessed at the time of transplant and at 100 days following ASCT using the International Myeloma Working Group (IMWG) Uniform Response Criteria.[74] Progression-free survival was measured from time of ASCT date to date of progression or clinical relapse. Overall survival was calculated from ASCT infusion date to date of death.

2.3.4 Statistical analysis

The $\chi 2$ test was used to compare proportions between groups. PFS and OS curves were plotted according to the Kaplan-Meier method and differences between curves were evaluated with log-rank tests. Univariate analysis of association of outcomes with risk factors was performed. These included conditioning dose (MEL140 vs MEL 200), age (<65 vs Ó65 yrs), International Staging Score (ISS), myeloma isotype, number of lines of treatment pre autologous transplant, treatment with prior novel agent therapy, response pre and post transplant and body surface area (BSA) <2m^2 vs Ó2m^2. P values <0.05 reflected statistical significance. Cox regression analysis was used to perform multivariate analysis for all variables with a P value of <0.1. Data was analysed using SPSS v22 (IBM, New York).

2.4 Results

2.4.1 Patients

A total of 193 patients were included in this retrospective study. 140 patients underwent MEL200 (median age 56 years, range 36-67) and 53 patients underwent MEL140 (median age 64 years, range 41-72).

Patient characteristics in the two treatment groups are shown in table 6. The MEL140 group included 15 patients dose-reduced on account of renal impairment. This likely explains the increased percentage of patients with light chain myeloma (LC-MM) and lower creatinine clearance in this treatment group. These patients all had an elevated ß2-microglobulin and as a result there was an increased proportion of patients with ISS stage 3 disease. The very nature of dividing patients into groups based upon melphalan dosing means that they are not and could never be equally matched. The necessity to dose reduce inherently reflects that those patients are either older, have renal impairment or have other comorbidities. This is important to note, because the non-inferiority of MEL140 compared to MEL200 is being assessed based upon the outcomes of two unequally matched groups.

There are a proportion of patients in each group with unknown ISS. This is because St Bartholomewõs is a referral centre for ASCT and as such, patients have frequently been diagnosed and treated at other hospitals prior to referral and diagnostic information was not always available. Similarly, cytogenetic data was unfortunately unavailable for a significant majority of patients as it was historically not performed as an upfront diagnostic test in most referral centres. The small proportion of patients in whom it was available would not have been of adequate size to draw any valid conclusions and so this data has not been collated.

Patients were dose reduced on account of renal impairment (n=15), age Ó65 years (n=23) and other comorbidities (n=15) (table 7). Patients with poor performance status either had a reduced Karnofsky score or a combination of chronic medical comorbidities.

Table 7: Reasons for dose reduction in MEL140 group

Reason for dose reduction	N
Renal impairment (CrCl<50ml/min)	11
Renal & cardiac impairment (EF<50%)	1
Renal & respiratory impairment (TLCO<50%)	2
Renal impairment & hepatitis C	1
Acute thromboembolism	2
TLCO <50%	1
Ejection fraction <50%	1
Poor performance status	11
Age >65	23

CrCl Creatinine clearance; EF Ejection fraction; TLCO Transfer factor of the lung for carbon monoxide

2.4.2 Response

Table 8 summarises the responses observed at D100 post ASCT. Despite the MEL200 group having a higher proportion of patients achieving CR prior to ASCT (24.3% vs 15% for

Table 8: Response at D100 post ASCT

Response	MEL200	MEL140	P value
CR(%)	61(44.5)	27(51.9)	0.554
VGPR(%)	20(14.6)	10(19.2)	0.311
PR(%)	54(39.4)	15(28.8)	0.096
RR(%) (≥PR)	134(98.5)	52(100)	-
SD(%)	2(1.5)	0	-

CR Complete response; VGPR Very good partial response; PR Partial response; RR response rate; SD stable disease

MEL200 and MEL140 respectively), there was no significant difference in CR post ASCT suggesting that the MEL140 group gained greater benefit in terms of CR conversion post transplant.

2.4.3 Clinical outcomes

Analysis of all patients demonstrated a median OS of 65 months with a 5 year OS of 57%. There was no significant difference in OS between the MEL200 and MEL140 groups; p=0.24 (figure 1). Median PFS for all patients was 20 months and again there was no significant difference between the two conditioning groups (figure 2).

Figure 1: Overall survival (MEL 200 vs MEL140)

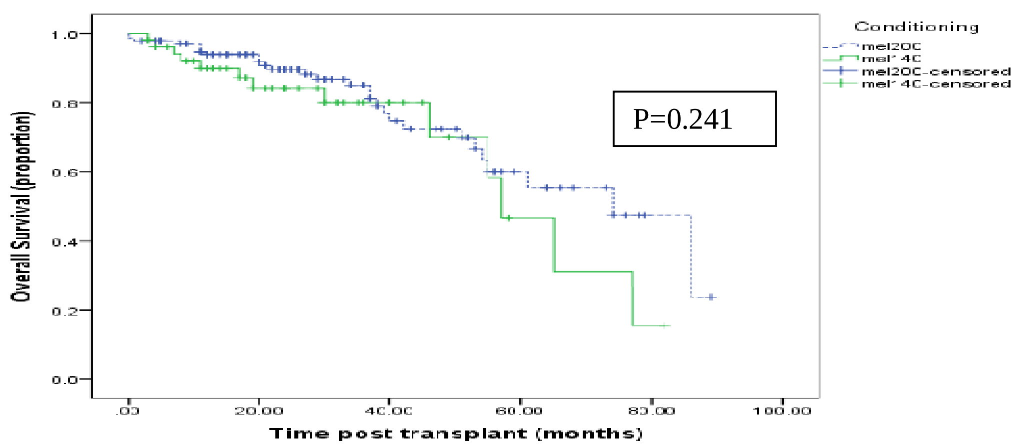
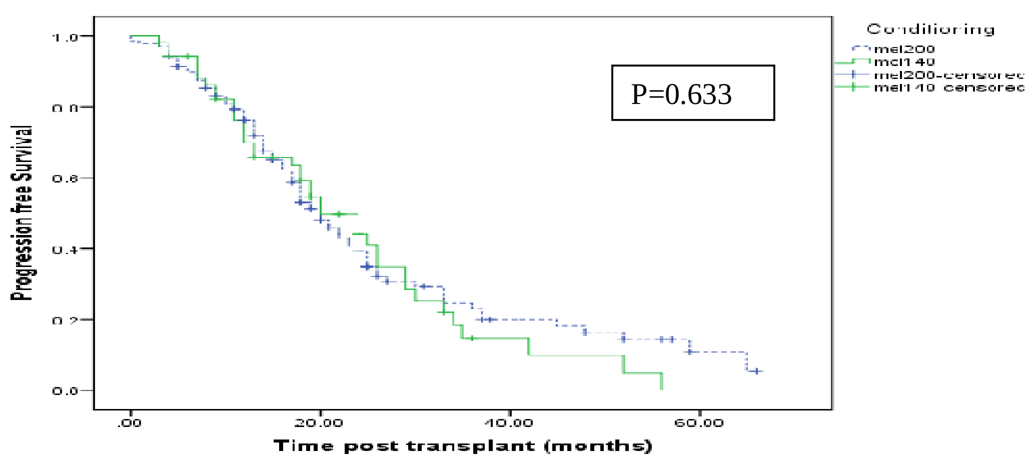
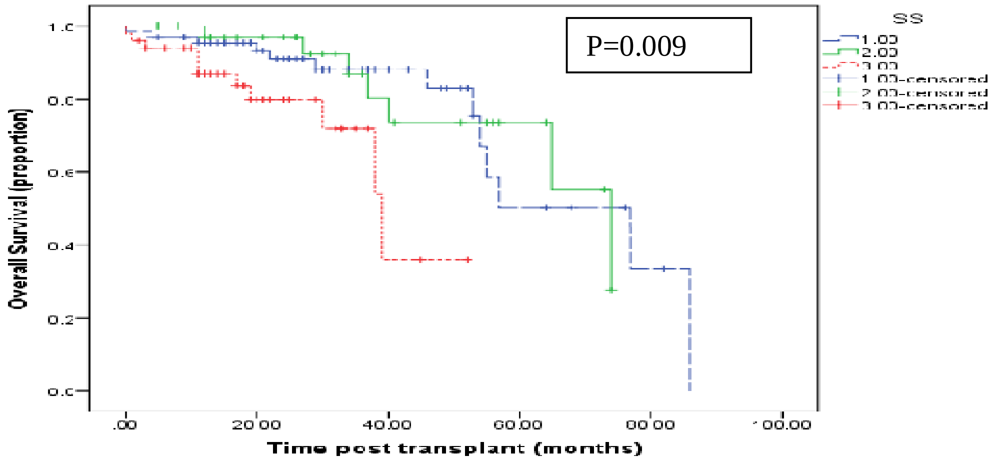


Figure 2: Progression free survival (MEL 200 vs MEL140)



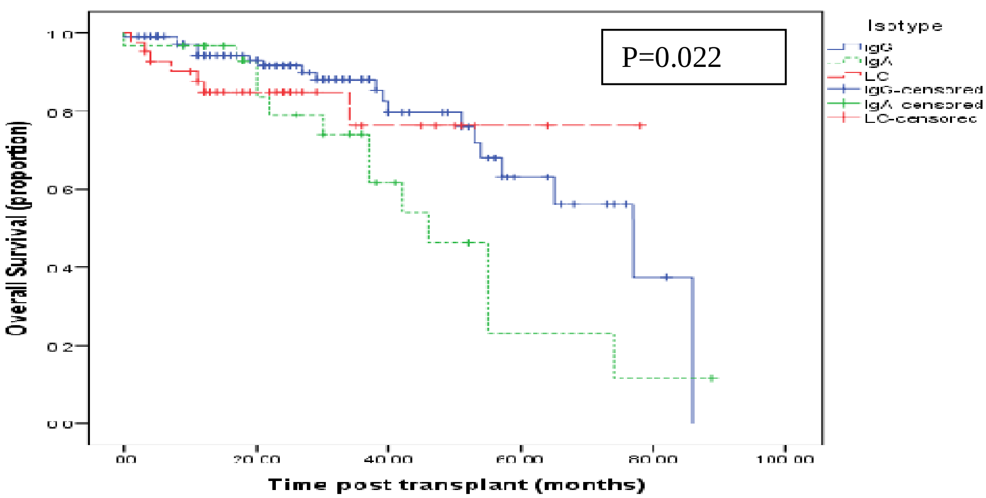
ISS was associated with a significant effect on OS across all patients with an ISS of 1, 2 and 3 resulting in a median OS of 77, 74 and 39 months respectively; p=0.009 (figure 3). ISS had no significant effect on PFS (p=0.55).

Figure 3: Overall survival of all myeloma ASCTs by ISS



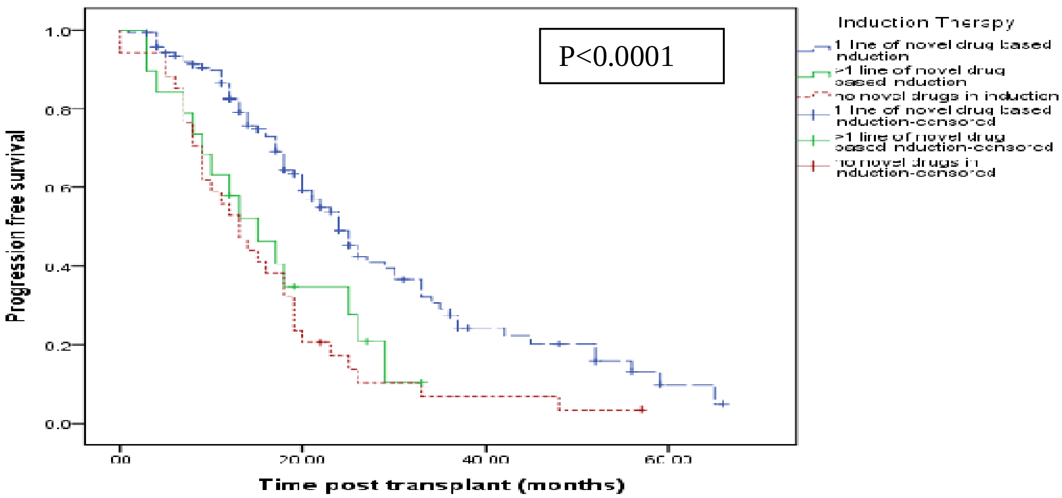
Myeloma isotype was also associated with a significant effect on OS (median OS IgG vs IgA was 77 and 47 months respectively; p=0.022, (figure 4). This was replicated in the MEL140 group, but in the MEL200 group, whilst the KM curve appeared similar, significance was not achieved. Isotype did not impact on PFS (p=0.823).

Figure 4: Overall survival in myeloma ASCTs by myeloma isotype



Prior induction treatment with novel agents demonstrated a trend to increased OS (median OS 74 vs 55 months; p=0.092) across all patients and within both conditioning subgroups. Median PFS in those treated with 1 line of novel agent-based induction, 2 or more lines of novel-agent based induction and no exposure to novel agents was 24,15 and 13 months respectively; p<0.0001(figure 5). This finding was replicated in both conditioning groups.

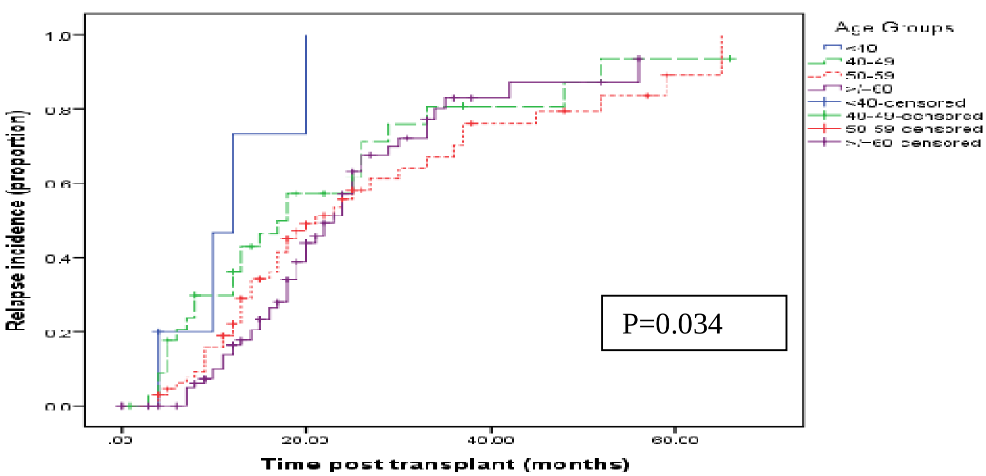
Figure 5: PFS in myeloma ASCT by induction therapy



Thirty-eight patients (19.6%) were equal to or over the age of 65. There was no significant difference in OS or PFS between patients <65 years and those Ó65 years across all patients (p=0.53 and 0.60 respectively). There was also no impact within each conditioning group, when using 65 years as a cut-off.

When patients were subdivided further, those under the age of 40 years (5 patients) had a median time to progression of 12 months which was significantly worse than the remaining cohort; p=0.034 (figure 6). However, with small patient numbers in this group this result should be interpreted with caution.

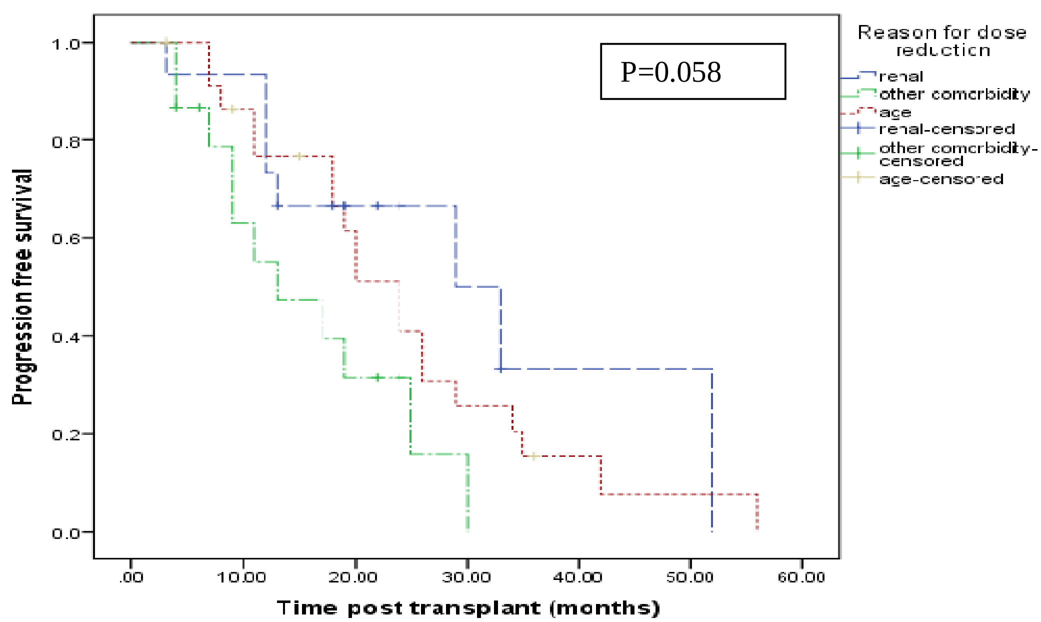
Figure 6: Relapse post ASCT by age groups



Univariate analysis demonstrated prior number of treatment lines, response pre-transplant and BSA did not impact on OS or PFS. Treatment response post ASCT did not impact on OS but did impact upon relapse outcomes. Those who achieved a CR had a significantly longer time to progression than any response inferior to this (24 vs 18 months respectively; p=0.049).

Within the MEL140 group, the reason for dose reduction (age, renal impairment or comorbidities) had no impact on OS (p=0.74). However, reason for dose reduction demonstrated a trend towards significance for PFS. Median PFS for patients dose reduced on account of renal impairment, age or comorbidity at 29, 24 and 13 months respectively; p=0.058 (figure 7).

Figure 7: PFS post MEL140 ASCT by indication for dose reduction



Cox regression analysis demonstrated isotype and ISS significantly impacted upon survival in all patients. Within the MEL200 cohort, induction therapy with novel agents was also significant whereas in the MEL140 group, only myeloma isotype was significant.

In order to try and further understand the impact of melphalan dosing, OS and PFS outcomes were further evaluated by body mass index (BMI) (average, overweight, obese), total melphalan dose in mg/kg and in those with a BSA of greater than or equal to 2m^2 the impact of capping the dose. 63 patients (32%) had a BSA of Ó2m^2. 13.5% and 19.2% of patients were overweight or obese by BMI criteria. In those with a BSAÓ 2m^2, 67% of patients were capped at a dose of $2m^2$. This proportion was the same in those above and below 65 years and within the two conditioning cohorts. There was significant variability in which patients where capped with no clear correlate in terms of BSA or BMI. However, none of these measures or interventions impacted upon progression or survival outcomes.

2.4.4 Toxicities

Three patients in the MEL200 group died of infective complications within 100 days of ASCT. Only 1 patient died in the MEL140 group. This resulted in a transplant related mortality (<100 days) of 1.75% and 2.11% for MEL140 and MEL200 respectively. Unfortunately, due to the retrospective nature of this analysis, the quality and availability of documentation regarding toxicity was inadequate in order to perform detailed and accurate toxicity analysis for side effects such as mucositis and infective complications. However, the length of stay in hospital was equivalent for the two treatment groups.

2.5 Discussion

Several studies have demonstrated that high-dose therapy with ASCT is superior to standard chemotherapy in patients <65 years. However, there has been no consensus to date on the most appropriate conditioning regimen for older patients or those with a reduced performance status. [50, 57-59] This retrospective analysis reports the findings of patients receiving either óstandardôMEL200 conditioning or MEL140 on account of renal impairment, age Ó65 years or the presence of comorbidities. There were no significant differences in PFS, OS or NRM between these two conditioning regimens. Therefore, in newly diagnosed patients with myeloma who fall into these adverse subgroups, ASCT with MEL140 is a viable and effective consolidation treatment strategy that appears to offer these patients similar survival benefit. The hypothesis that those undergoing MEL140 would have inferior outcomes has therefore not been supported. This finding is particularly striking given that the MEL140 cohort of patients were, by definition, a worse group in terms of their performance status and expected outcomes.

In the treatment of myeloma today, most patients will receive multiple lines of therapy and classically, each with result in a shorter PFS than the previous one. The measurement of overall survival as an outcome measure of one line of treatment is therefore of limited value as clearly effects on OS will be multifactorial. PFS is a much more useful outcome measure to assess the impact of ASCT alone and it is therefore of great importance to identify that median PFS was exactly the same in the two conditioning groups at 20 months. PFS2, the time to second objective disease progression on next-line treatment or death from any cause, is becoming increasingly important as an outcome measure. A respectable PFS may hide the impact of a treatment on the tumour's drug resistance profile or indeed the patient's ability to tolerate further lines of treatment. It would have been very useful to use PFS2 to identify what the impact of MEL140 was in terms of this group's ability to receive and tolerate further lines of treatment. However, because a significant proportion of patients received their post transplant therapies back at their referring hospital, this data was unavailable.

This was a non-randomised retrospective study and as such clinician choice in selecting which patients proceeded with ASCT was undoubtedly a factor in the equally successful results achieved by MEL140. Rather than using an arbitrary age cut-off, a more considered clinical approach evaluating age, along with the presence of comorbidities and performance status, should be taken when deciding who should undergo ASCT and with which conditioning regimen. This is particularly important in the absence of data suggesting that myeloma is biologically different in the older population[75, 76] and that differences in outcome in the older patient population are more likely related to increased comorbidities and poor performance status. It has been reported that the presence of comorbidity in itself does not directly correlate with functional ability in older patients with malignancies.[77] Therefore, this should not serve as a deterrent to ASCT and patients should be considered on an individual basis regarding their fitness to proceed to high-dose therapy. There may well be a referral bias, potentially resulting in under-treatment with ASCT in patients aged >65 years, who are otherwise fit to receive intensive therapy. This study highlights the benefits that may be achieved in this population group.

As melphalan is renally excreted, there is concern regarding the risk of prolonged bone marrow suppression in those with renal impairment. In view of the increased toxicities reported when full doses were given to patients with renal impairment, dose reductions have been recommended.[61, 78] Although the rationale for dose reduction is clearly different, in order to produce an adequately-sized patient cohort, with which to be able to make valid conclusions, all indications for dose reduction were grouped together. However, the three indications for dose reduction (age, comorbidity and renal impairment) were also analysed separately. Whilst indication had no impact on OS, there was a trend towards significance for PFS. Those dose-reduced on account of renal impairment had the longest PFS, with a median of 29 months. This is actually very impressive and is significantly longer than the PFS in the entire MEL200 cohort. Those dose-reduced on account of age had a median PFS of 24 months, in line with findings in the MEL200 cohort. Several studies have supported the use of ASCT in older patients and EBMT data has reported that the proportion of patients above the age of 65 has increased from 3% in 1991 to 18% between 2006 and 2010.[79] This analysis supports that finding with 19.6% of patients in this study being over the age of 65. It further validates the viability of ASCT in patients in this age group and unlike any other study has demonstrated the efficacy of a lower-dose conditioning regimen in this population.

Those with comorbidities had a median PFS of 13 months. It is important to remember that this is a cohort of patients that were not considered fit enough to tolerate MEL200. If they had not received MEL140, they would not have undergone ASCT. Although no direct comparison to a non ASCT strategy was performed in this analysis, the survival outcomes of this subgroup may still be superior to a non transplant approach and this would be in interesting and important area of future of work. Furthermore, whilst they may progress sooner, their OS is not inferior to the remainder of the cohort. Therefore whilst recognising that there is an inferior progression-free survival, the MEL140 approach should be considered an appropriate treatment strategy in this cohort.

The impact of ISS on OS was validated again in this study, with those with an ISS of 3 having particularly inferior outcomes.[80] Despite the MEL140 cohort having a larger proportion of patients with ISS 3, the impact of ISS on OS was not seen in this cohort. This is most likely due to patient numbers, and shorter follow-up time for this subset of patients as median OS was yet to be reached.

A significantly longer time to progression was seen in those who achieved a CR post ASCT, although an impact on survival was not seen in this analysis. There is increasing evidence that achieving a complete remission post ASCT correlates with increased OS and PFS.[81-85] The increased conversion rate of patients achieving CR observed in the MEL140 group (14.5% pre-ASCT to 47.3% post ASCT) is therefore a potentially important finding. This observation suggests that this may well translate into an improved long-term OS in this population group, who would likely have fared poorly without ASCT.

The treatment of myeloma has been revolutionised over the last decade by the advent of ónovel agentsô These include proteasome inhibitors such as bortezomib (Velcade) and immunomodulatory agents (IMiDs) including thalidomide, lenalidomide (Revlimid) and Pomalidomide (Imnovid). These drugs can be used in patients of all ages in the outpatient setting. Their arrival has significantly improved survival outcomes in patients with myeloma.[86] In this analysis, their use in induction prior to ASCT resulted in improved PFS with a trend to increased OS. The role and timing of ASCT in the era of novel agents, remains unclear. However, in this analysis, 81.4% of patients treated with MEL200 and 92.7% of MEL140 patients were treated with novel-agent-based induction therapy. Therefore, the findings of this study are applicable to the novel-agent era and support the practice of dose-reduced ASCT in those deemed unsuitable to receive the MEL200 standard.

The observation that patients under the age of 40 have shorter times to progression is not supported by the literature and is likely to be the consequence of small numbers. [87, 88] The IMWG evaluated 10,549 patients and identified that those younger than 50 years had no differences in cytogenetic profile, tended to present with a lower ISS and demonstrated better OS.[89, 90]

Unfortunately pharmacokinetic data was unavailable in this retrospective analysis and so whilst recognising the clinical non-inferiority of MEL140, there is little understanding of exactly what the difference is between MEL200 and MEL140 at a cellular level. There is some recognition that there may be a genetic component to a patient's melphalan metabolism and its consequent effects. However, to date there is no definitive marker for testing. There is also limited evidence on how best to approach dosing in those patients with an increased BSA. There was no association identified with BSA, BMI, dose capping or total melphalan dose with survival outcomes. Similar studies have also identified a lack of uniformity in the dosing strategy used in patients with a raised BSA but confirmed that this cohort of patients do not have inferior survival outcomes and that this should not be a deterrant to proceed to ASCT.[91] The most recent consensus guidelines have suggested that patients should not be capped and dosing should be based upon actual weight.[92] Given that no differences were seen in the capped vs uncapped group, this data would support those recommendations.

In conclusion, MEL140 is a safe and effective conditioning regimen for older patients, those with renal impairment or other co-morbidities. The outcomes achieved by the MEL140 cohort are particularly striking because the patients conditioned with MEL140 had biological or clinical features that would, intuitively, result in worse outcomes. These observations suggest that the benefits of MEL200 and MEL140 may be equivalent and that the hypothesis of this work was not supported.

A prospective clinical trial between MEL200 and MEL140 would be required to prove this definitively and recommendations for changing the standard of care cannot be made on the basis of these results alone. However, based upon my findings, I would suggest that the BSBMT recommendations could be modified, with a statement that the decision to transplant patients aged between 65 and 70 years should be based upon a full clinical assessment evaluating the presence of comorbidities and performance status rather than an arbitrary age cut-off and that consideration may be given to dose reduction in cases where the patient is not considered fit to receive standard MEL200.

CHAPTER THREE

Allogeneic Stem Cell Transplantation

In Multiple Myeloma

3.1 Introduction

The role of allogeneic stem cell transplantation in myeloma has been an area of controversy for over 25 years. Investigators in the late 1980s were excited about the curative potential an allograft could offer with the use of myeloablative conditioning. Indeed, small cohorts of long-term survivors were identified using this approach.[93] However, this was offset by an unacceptably high NRM in the order of 50%[94, 95] predominantly secondary to infection and GVHD and organ toxicity and consequently, MAC allografts in myeloma were largely abandoned.

The advent of RIC-SCT reintroduced allogeneic transplantation as a treatment option. This approach relied less on the high dose chemotherapy and radiotherapy that was utilised in ablative transplants to eradicate myeloma cells. Instead, it focused on the graft-vs-myeloma effect which had been proven by the success of DLI in patients who had not responded or had progressed post transplant.[96, 97] Offering less toxicity and consequently a significantly reduced TRM compared to ablative conditioning, RIC-SCT widened the cohort of patients that could potentially undergo an allogeneic transplant. [37]

An EBMT review analysed data on 229 patients with myeloma who had undergone a RIC-SCT.[98] This included 28 different conditioning regimens, predominantly fludarabine-based (96%) with additional melphalan, TBI, busulphan, cyclophosphamide. Furthermore, 59% were T-cell depleted regimens with either Alemtuzumab, an anti-CD52 antibody, or anti-thymocyte globulin (ATG). OS and PFS at 3 years was 40.6% and 21% respectively. D100 TRM was 10%, increasing to 26% at 2 years. The majority of deaths were secondary to infection and GVHD.

Further registry data analyses have compared RIC to MAC and concluded that whilst the RIC approach undoubtedly offered a significantly reduced TRM, there was no overall survival benefit because it also carried a significantly increased relapse rate.[99]

Autologous stem cell transplantation is the standard of care following induction therapy in young fit patients with newly diagnosed myeloma.[50, 57, 58] However, there is no survival plateau with this approach. Consequently, there have been several prospective studies comparing a tandem autologous transplant approach to autologous transplant followed by a RIC-SCT in those with a matched sibling donor.[100-106] The studies varied in their design making comparison difficult. Garban et al only included patients deemed ‘high risk’ and used a T deplete approach with ATG conditioning. The remainder were more inclusive in their patient cohort and used either TBI alone,[101, 103, 104] TBI and fludarabine[106] or the more intensive fludarabine and melphlalan conditioning regimen.[102] The autologous arms also varied; whilst most studies used the now standard melphalan conditioning, melphalan doses differed amongst the studies as did the use of maintenance therapy post autograft.

Whilst some of the above studies concluded that PFS and OS were equal using the two approaches, the Italian group and EBMT analysis demonstrated that the auto-allo approach resulted in an overall survival advantage. It was universally, and unsurprisingly, demonstrated that the auto-allo approach had a significantly higher TRM than the tandem autologous approach (cumulative TRM 10-17% vs 1-5% for tandem autograft) although this level of TRM is much more favourable than the data from the ablative era.

The TRM associated with a RIC-SCT seems unjustified to some clinicians in an era where upfront novel agent therapy can potentially offer several years of progression-free survival. As a result, the role of RIC allografting in patients with relapsed myeloma has been investigated more recently. Whilst patients with progressive myeloma undoubtedly have more treatment options available to them since the advent of novel agents, their prognosis remains poor. There is consequently an argument that the increased TRM associated with allogeneic transplantation is perhaps more justified in this setting.

Retrospective studies have been performed in patients who have relapsed and have demonstrated it to be a viable treatment option. However, its cumulative TRM is 20-30% at 3 years which is higher than studies where allogeneic transplant was performed at first response.[107] In studies that performed a donor vs no-donor analysis comparing the outcome of patients undergoing a RIC with those undergoing salvage with novel agents, the transplant arm has shown superior PFS but no OS benefit.[108, 109]

The BSBMT guidelines have recommended that for patients in first response with a sibling donor, an allogeneic transplant is óstandard of careô (table 5). However, not all patients in first response with an identified sibling donor undergo an allogeneic transplant and it makes no recommendations about patient selection. For those patients in first response with a matched unrelated donor, or in patients who have relapsed, allogeneic transplantation is listed as a óclinical optionô

With the role of an allograft still highly debated and conclusive evidence for there being an overall survival benefit still lacking, there remains a large question mark over which patients should be offered an allogeneic transplant and when. Patient selection and timing of the transplant remains at the discretion of the treating physician. The International Myeloma Working Group consensus statement has recommended that in the absence of convincing evidence for a survival benefit that allogeneic transplantation should only be performed in the context of a clinical trial.[110]

It seems reasonably clear that in order to determine whether allogeneic transplantation really has a place, the key is to identify which patients are most likely to benefit from it. At St Bartholomewõs, a variety of patients have undergone allogeneic transplantation for myeloma. Practice has included transplantation of patients with all ISS scores and transplants have been performed both at first response and at relapse. Centre practice has been to offer non-ablative conditioning with fludarabine and cyclophosphamide. This low-intensity regimen has been demonstrated to have minimal toxicity whilst still achieving equivalent disease outcomes in other settings.[111, 112] I have developed the hypothesis below in order to challenge perhaps the most controversial statement surrounding allogeneic transplantation in the BSBMT indications table. In addition, this retrospective analysis aims to assess the outcomes of allogeneic transplantation in patients with myeloma and attempt to identify which patients benefit most from it.

3.2 Hypothesis

The outcome after allogeneic transplantation for patients with multiple myeloma justifies the increased toxicity by decreasing relapse.

3.3 Methods

3.3.1 Patient eligibility

A total of 35 patients with multiple myeloma underwent RIC-SCT between 1999 and 2011 at St Bartholomewõs. They included patients of all ISS scores and patients who were transplanted both in first response and at relapse. Written informed consent was obtained from all patients and donors in accordance with the Declaration of Helsinki.

3.3.2 Treatment protocol

Transplant conditioning was with fludarabine 25 mg/m^2 on Dī 6 to Dī 2 and cyclophosphamide 1 g/m^2 on Dī 3 and Dī 2. In one case, the donor was a 1A mismatch and the recipient received Alemtuzumab in addition. Methotrexate (5 mg/m^2 on D+1,D+3 and D+6), together with ciclosporin (5 mg/kg on Dī 2 then 3 mg/kg per day from Dī 1, tailing at day +100 or sooner in the presence of mixed chimerism) was used for GVHD prophylaxis.

Trough CSA levels were measured in whole blood twice weekly post-transplant. CSA was dosed to maintain levels between 150ï 300mcg/L.

3.3.3 Supportive care

All patients received antifungal and antimicrobial prophylaxis with fluconazole and ciprofloxacin until neutrophil engraftment. All patients were commenced on cotrimoxazole following engraftment as prophylaxis against *Pneumocystis jiroveci*. All patients received antiviral prophylaxis with aciclovir for a minimum of 6 months or longer if still on immunosuppression. Peripheral blood samples were monitored weekly for CMV re-activation by quantitative polymerase chain reaction (PCR). Patients with evidence of CMV reactivation were treated with pre-emptive valganciclovir.

3.3.4 Patient evaluation

Disease response was assessed at the time of transplant and at 100 days following HSCT using the IMWG Uniform Response Criteria.[74] PFS was measured from time of SCT date to date of progression or clinical relapse. OS was calculated from SCT infusion date to date of death. NRM was defined as death in the absence of disease relapse or persistence. Acute GVHD was diagnosed and graded using current consensus criteria.[113] Chronic GvHD was diagnosed and scored using Seattle criteria.[114]

3.3.5 Statistical analysis

Progression-free-survival and overall survival curves were plotted according to the Kaplan-Meier method and differences between curves were evaluated with log-rank tests. Univariate analysis of association of outcomes with risk factors was performed. These included age, gender, haematopoietic cell transplant comorbidity index (HCT-CI), ISS, myeloma isotype, number of lines of prior treatment, prior novel agent therapy, response pre transplant, donor type, risk of CMV reactivation, major ABO mismatch, gender mismatch, and the occurrence of acute and chronic GVHD. P values <0.05 reflected statistical significance. Cox regression analysis was used to perform multivariate analysis on all factors with a P value of less than 0.1.

3.4 Results

3.4.1 Patient characteristics

A total of 35 patients with multiple myeloma underwent RIC-SCT between 1999 and 2011. Patient characteristics are listed in table 9. 82% patients were male and the median age at the time of transplant was 52 years (range 33-63). 74% had a matched sibling or other family member donor and with 1 exception, a 1A mismatched VUD, the remainder had matched unrelated donors. Peripheral blood was the stem cell source in all cases. 11, 6 and 10 patients had an ISS of 1, 2 and 3 respectively at diagnosis (data was unavailable on the remainder). 35%, 45% and 20% of patients had a HCT-CI of low, intermediate and high respectively.

Table 9: Patient characteristics undergoing RIC-SCT for myeloma

Total no. of patients		**35**
Male sex (%)		29(82.9)
Age (median)[range]		52 [33-63]
Myeloma Isotype	**IgG (%)**	22(62.9)
	IgA(%)	6(17.1)
	Light chain myeloma(%)	2(5.7)
	Non-secretory(%)	1(2.9)
	IgD (%)	1(2.9)
	Not known(%)	3(8.6)
International Staging Score (ISS)	**1(%)**	11(31.4)
	2 (%)	6(17.1)
	3 (%)	10(28.6)
	Not known (%)	8(22.9)
Previous autologous transplant	**Yes(%)**	24(68.6)
	No(%)	6(17.1)
	Not Known(%)	5(14.3)
Pre transplant novel therapy	**Yes(%)**	16(45.7)
	No(%)	14(40)
	Not known(%)	5(14.3)
Pre transplant response status	**CR1(%)**	10(28.6)
	CR2(%)	3(8.6)
	PR1(%)	14.1(40)
	PR2(%)	3(8.6)
	Not known(%)	5(14.3)
Donor type	**Matched sibling**	24 (68.6)
	Matched VUD	8(22.8)
	Mismatched VUD	1 (2.9)
	Other (matched other family member)	2(5.7)

The patient group was heterogeneous. Two patients failed to harvest stem cells and had a RIC-SCT in first response, directly following induction chemotherapy. Ten patients had a tandem autologous-allogeneic approach having achieved a CR following autograft. Eleven patients received maintenance or further chemotherapy following their initial autograft to improve response, prior to proceeding with RIC-SCT. Four patients had their RIC-SCT following relapse and a second autograft and two had it directly following relapse, having failed to harvest cells for a second autograft. There was no data on the remainder.

3.4.2 Engraftment and chimerism

Patients received a median $CD34^+$ cell dose of 6.05 x 10^6/kg (range 2.94-15.01) and median $CD3^+$ T cell dose of 2.51 x 10^8/kg (range 0.5-15.7). Median time to neutrophil engraftment was 15 days (range 12-24). There were no cases of engraftment failure. Full donor chimerism (FDC) on whole blood was achieved in all assessable patients at a median time of 3 months.

3.4.3 Non-relapse mortality and GVHD

100 day NRM was 5% (n=2); both secondary to infection. Cumulative 1 year NRM was 11% (n=4) with 1 patient having a GVHD-related death and the other dying in an accident. 3 year NRM remained at 11%. Acute GVHD (grade II-IV) occurred in 11.4% of cases. Chronic GVHD requiring systemic immunosuppression occurred in 62.1% of all evaluable patients.

3.4.4 Response and outcome data

Median follow-up of surviving patients was 80 months. Best responses post allograft were 72% complete response (CR) and 28% partial response (PR) from all evaluable cases (17% data unavailable). Median OS from the time of transplant for all patients was 65 months (figure 8) with a 5 year OS of 57%. Median PFS was 29 months (figure 9).

All relapses occurred by 41 months post transplant. Beyond this time point, a plateau was evident with 30% of patients not relapsing post transplant. A similar plateau is present but less clearly evident on the OS graph because some relatively early relapses manifested as significantly later disease-related deaths as patients were treated with further lines of treatment post transplant. One patient relapsed at 17 months post transplant and died 143 months post transplant (figure 8).

Univariate analysis demonstrated that patient gender, myeloma isotype, ISS, HCT-CI, donor type, previous autologous transplant, pre transplant response (CR vs <CR) and induction

Figure 8: Overall survival in myeloma RIC-SCT

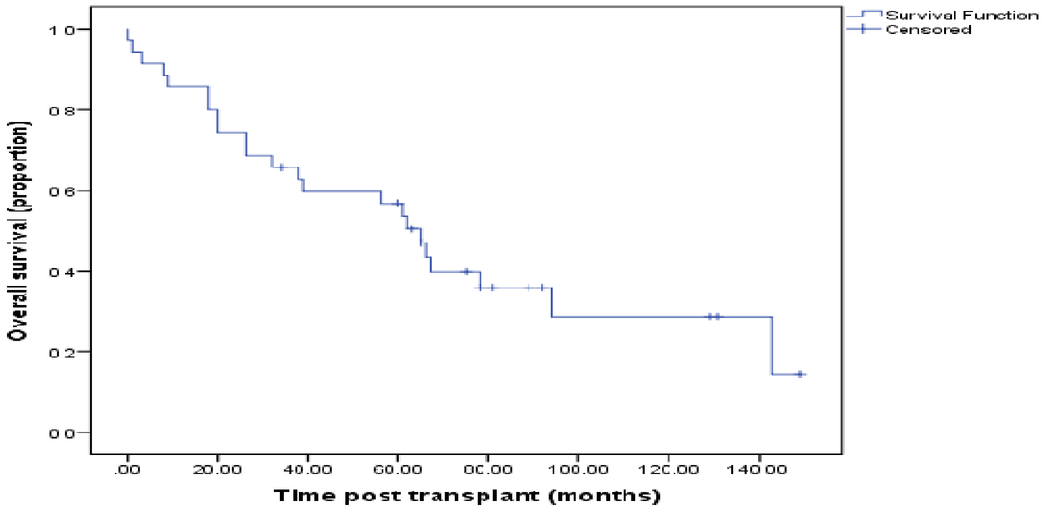
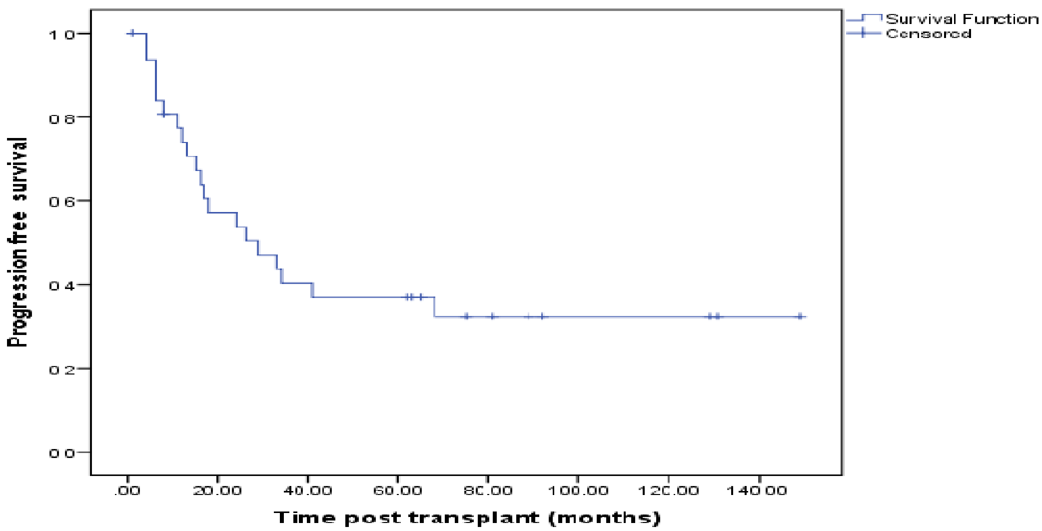


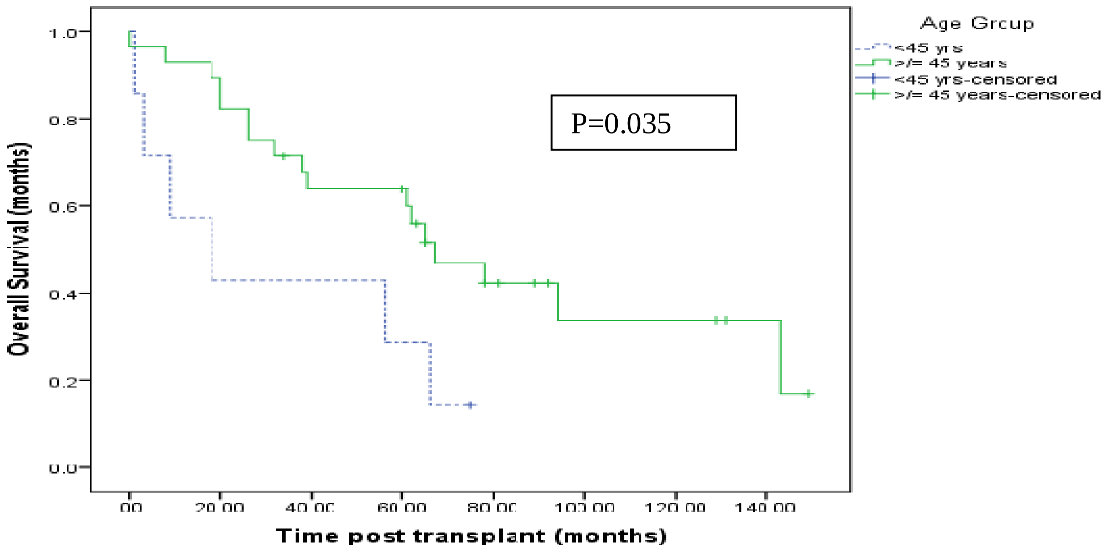
Figure 9: Progression free survival in myeloma RIC-SCT



with novel agents compared to older regimens prior to transplant had no effect on PFS or OS.The presence of gender mismatch (male recipient with female donor), major blood group mismatch and presence of CMV reactivation risk (donor or recipient or both CMV IgG positive) also had no impact on PFS and OS.

There were 7 patients under the age of 45 years. This cohort had a significantly worse OS compared to the remainder of the cohort with a median OS of 18 months vs 67 months; p=0.035 (figure 10). However, this is likely to be the effect of small sample size and this finding should be interpreted with caution. Age had no impact on relapse incidence.

Figure 10: Impact of patient age on OS in myeloma RIC-SCT



Number of prior treatment lines had a significant impact on both OS and PFS. Patients who had received 1 or 2 prior therapies had a median OS of 78 months compared to 26 months in those who had received 3 or 4; p=0.018 (figure 11). Patients who had 1 or 2 prior therapies had a median PFS of 34 months compared to 13 months in those who had received 3 or 4; p=0.013 (figure 12). Prior number of treatment lines remained significant in multivariate analysis.

Figure 11: Effect of number of prior treatment lines on OS in myeloma RIC-SCT

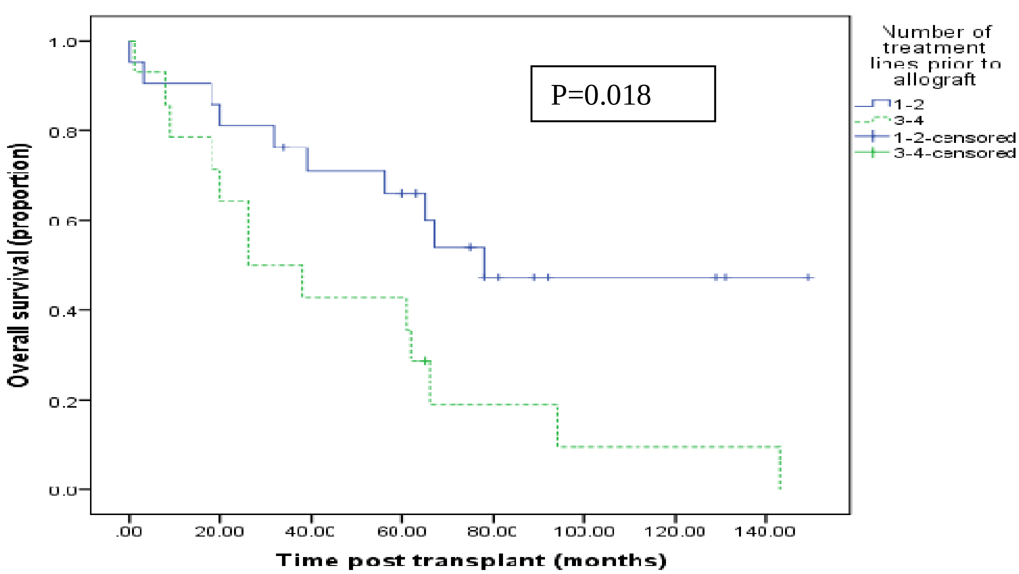
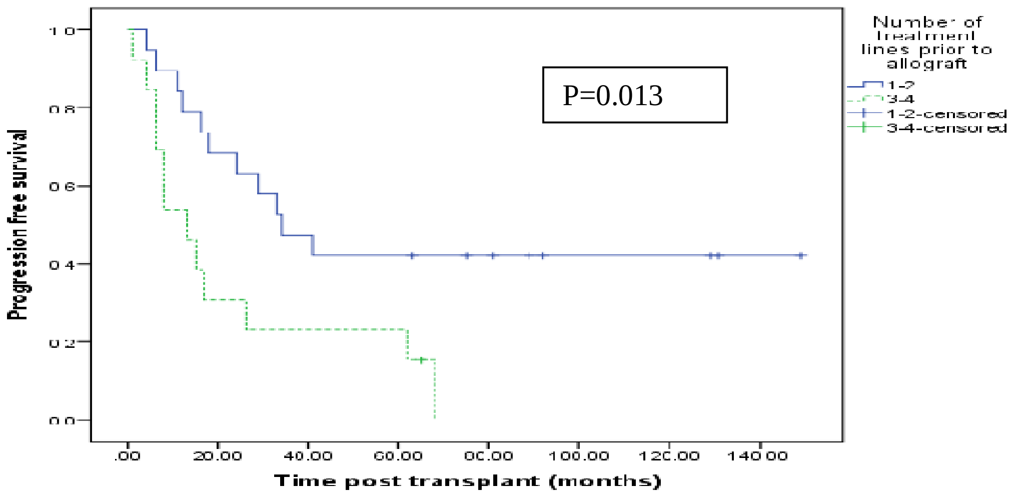


Figure 12: Effect of number of prior treatment lines on PFS in myeloma RIC-SCT



Four patients (13.3%) developed acute GVHD grades II-IV and 62% of patients developed chronic GVHD. The occurrence of acute GVHD had no effect on PFS or OS, although with only 4 cases, an effect would have been difficult to detect. The occurrence of chronic GVHD demonstrated a trend towards an improved PFS; p=0.071 (figure 13). Median OS in those with and without chronic GVHD was 94 months and 62 months respectively; p=0.194 (figure 14). The effect of chronic GVHD on both PFS and OS, may have been strengthened with larger patient numbers.

Figure 13: Effect of chronic GVHD on PFS in myeloma RIC-SCT

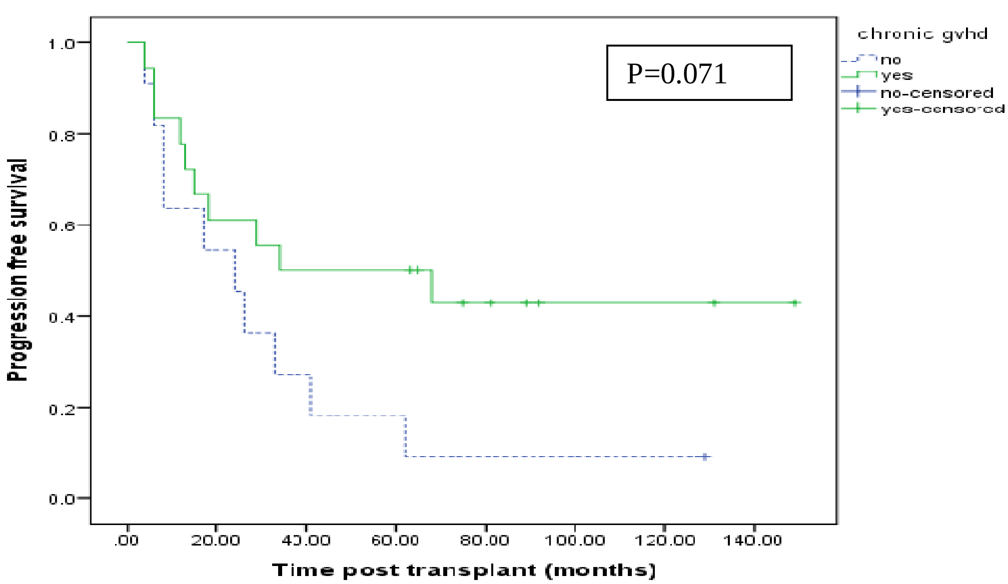
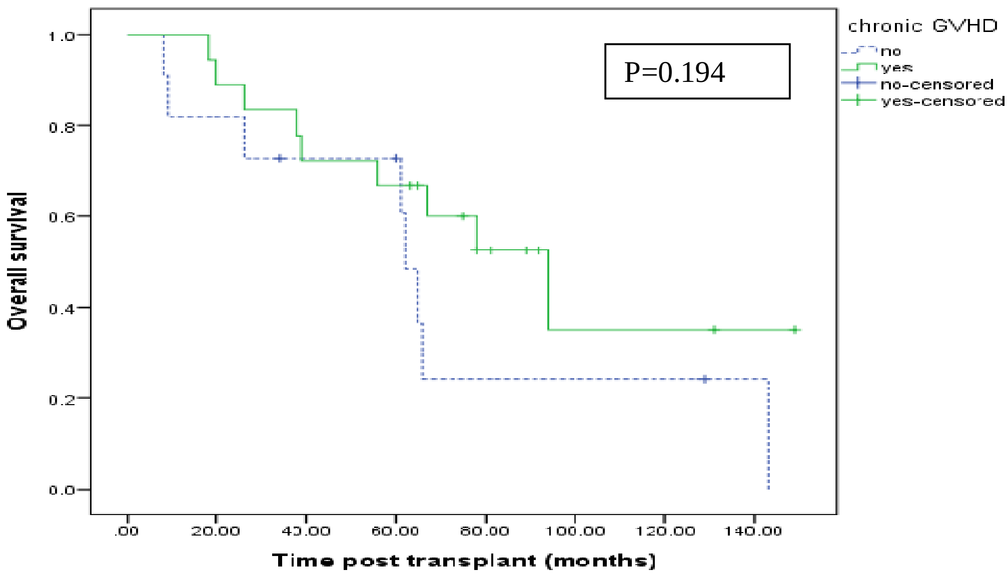


Figure 14: Effect of chronic GVHD on OS in myeloma RIC-SCT



Four patients received DLI; three for disease progression all of whom died within the following 12 months. One received DLI for low T-cell chimerism. This was followed by the development of full donor chimerism and the onset of GVHD 1 month later, but disease relapse still occurred 14 months later. The patient survived for 73 months post transplant, but ultimately died of disease progression.

3.5 Discussion

There is long-standing controversy surrounding the place of allogeneic transplantation in myeloma, with no convincing evidence to date for a survival benefit.[110] Furthermore, there is ongoing debate about the type of conditioning, whether to transplant upfront or at relapse and whether it should be reserved solely for those with high risk disease.

This is a retrospective analysis of 35 patients receiving fludarabine and cyclophosphamide conditioning as part of a non-ablative allograft. Median OS and PFS were 65 and 29 months respectively. This is in line with other reported studies where median OS has been reported at 49-79 months and PFS at 19-30 months.[101, 102, 104]

Median OS and PFS for upfront autologous transplantation (MEL200) at St Bartholomew's is 65 and 20 months respectively. These are clearly different groups of patients and direct comparison cannot be made. The survival assessment is not made from equivalent time-points in the two cohorts and the allograft cohort consists of more heavily pre-treated patients with a sizeable proportion transplanted at relapse. However, it might suggest that there is unlikely to be a significant *short-term* overall survival benefit to be gained by having an allogeneic transplant when the cohort is considered as a whole.

The key theoretical difference between the two transplant approaches is that reduced-intensity allogeneic transplantation, through its graft-vs-myeloma effect, should produce a plateau in OS, so that a proportion of those transplanted will be long-term survivors who are cured from their myeloma. In this analysis, a plateau was evident in relapse incidence with 30% of patients progression-free beyond 5 years. Median time to death following relapse/ progression was 23 months (range 4-125 months).

Unlike many other haematological malignancies where relapse post allogeneic transplant carries a dismal outcome with few treatment options available, this study serves to demonstrate that for patients with myeloma this is not the case. This data demonstrates that despite relapse following allogeneic transplant, patients may go on to receive and potentially have long responses to combination chemotherapy and leads to consideration of what the definition of treatment failure is in this scenario. PFS2 data in this setting would therefore be very instructive in order to understand what the impact of allogeneic transplantation is on consequent therapies for those who do relapse. Unfortunately, because of the retrospective nature of this analysis, many patients received subsequent therapies at other centres and therefore accurate data was not readily available to generate this data.

This study has shown a cumulative NRM of 11% at 3 years and is therefore significantly lower than the 26% reported by the EBMT review.[98] Almost 60% of patients in that review had T deplete conditioning with a significantly reduced occurrence of chronic GVHD (71% vs 39%). They reported that patients who had undergone T cell depletion had a significantly increased NRM, likely related to infection. In addition, they had a reduced time to progression and OS, most likely a reflection of the reduced graft-vs-myeloma effect. Our NRM even compares favourably to studies reporting patients solely treated with upfront auto-allo approach (NRM 11-17%).[102-104, 106] Considering that this patient cohort includes patients at relapse in whom NRM in allogeneic transplantation is higher,[107] this study demonstrates that T replete conditioning with fludarabine and cyclophosphamide has minimal toxicity when compared to many other conditioning regimens.

Sixty-two percent of patients had chronic GVHD which, again, is in line with other studies. Despite the high incidence of GVHD, there was only one GVHD related death, providing further support for the use of a T replete platform. The beneficial effect of chronic GVHD on both PFS and OS may have been strengthened with larger patient numbers but was not detected at significance in this analysis. The use of DLI was ineffective in achieving disease control in all cases. All patients achieved FDC, with a significant proportion doing so following the tailing and cessation of CSA. Despite this, a significant proportion of patients went on to develop progressive disease. Evidence of the graft-vs-myeloma effect in this analysis is therefore limited.

Some of the controversy surrounding the role of allogeneic transplantation in myeloma is related to the improving outcomes being achieved with novel therapies such as bortezomib, thalidomide and lenalidomide.[115] Only one prospective study looking at the auto-allo approach included patients who had received novel agents (thalidomide) as part of their induction.[105] Their outcomes were comparable to studies using older induction regimens. Our study identified no difference in OS or PFS in patients who had received novel agents prior to allogeneic transplantation. However, both this study and those already reported, are likely to be confounded by the fact that patients who received their induction/ treatment prior to transplant in the pre-novel agent era, will undoubtedly have received these drugs at the time of relapse or progression, making interpretation of overall survival in this context difficult.

A weakness of this retrospective analysis was the patient heterogeneity. Patients had been treated with several different approaches prior to allogeneic transplant. This resulted in several groups utilising different treatment pathways, each with small numbers, making it difficult to compare outcomes with any validity. Auner et al reported that OS was better in those who had only had 1 previous autograft.[116] In this study, receiving 1-2 lines of treatment resulted in significantly superior OS and PFS compared to those who had received 3 or 4. This is likely to reflect the evolution of more treatment-resistant disease. There were only 3 patients identified to be in CR2 and three patients in PR2. Perhaps if larger numbers were available, the impact of treating upfront versus at relapse would have been more evident. Whilst lines of treatment do not equate completely with treatment in first response or at relapse, it does suggest that perhaps treating patients at relapse (who have usually had more lines of therapy) may be associated with worse outcomes, consistent with previous reports.[107] Again, PFS2 data would be helpful to evaluate this area further.

Allogeneic transplantation has the potential to induce a much higher proportion of CRs compared to autografting.[117, 118] In this study, 40% of patients were in CR prior to transplant which rose to 72% following the procedure. In autologous transplantation, it has now been clearly demonstrated that those patients who achieve a CR both prior to and post transplant have a superior PFS & OS.[82, 119] However, in this study, achieving a CR vs PR pre-allo SCT had no significant effect on OS or PFS.

There was no impact of ISS on PFS or OS. Unfortunately cytogenetic data was not available on the majority of the patients who had initially been diagnosed and treated at other centres. Previous studies in which patients were stratified by risk, used criteria such as high β2M and chromosome 13 deletions by FISH. They reported that those with *del*13q14 had a worse EFS and OS.[120] Whilst β2M is still relevant (in combination with presentation albumin) for prognostication,[80] chromosome 13 abnormalities in isolation are no longer considered predictive of outcome and instead numerous other cytogenetic abnormalities have been identified, which allow us to classify patients as high risk today.[121, 122] Schilling et al suggested that the negative impact of t(4;14) could be overcome by allogeneic transplantation and whilst this group found that patients with *del*(17p) still fared poorly,[123] Roos-weil et al demonstrated that patients with both t(4;14) and *del*(17p) demonstrated no difference in EFS & OS, supporting the suggestion that allogeneic transplantation may allow these patients to overcome their poor risk.[124]

Patients with 'ultra-high risk myeloma' have an estimated median survival of less than 24 months utilising conventional therapies and ASCT. They comprise patients with ISS 3 and poor risk genetic abnormalities.[125] This cohort of patients with ultra-high risk are increasingly seen as appropriate allogeneic transplant candidates in view of their otherwise poor outcome. The policy for patient selection at St Bartholomew's has now changed to include consideration of those patients who are ultra-high risk and who have a suitable donor.

This analysis unfortunately has several limitations. It was a non-randomised retrospective study and as such clinician choice in selecting which patients underwent allogeneic transplantation undoubtedly had an impact on outcomes. There were no clearly defined criteria regarding patient selection and as such the wide variety of patients made analysis difficult. A formal HCT-CI assessment was not performed prior to transplant and patient selection was at the clinician's discretion. Logically, to offer a relatively controversial treatment choice with a significant risk of mortality in comparison to other myeloma therapies, it would be expected that only patients who were considered fit would have been considered appropriate. However, this retrospective analysis demonstrated that patients with a full range of HCT-CI scores were included, including 20% who were in the 'high' category. However, despite this HCT-CI had no effect on PFS or OS and despite the heterogeneous population, the low NRM with this approach has already been highlighted.

St Bartholomewõs is one of the largest UK centres performing allogeneic transplants in myeloma. However, with only 35 allogeneic transplants performed over a 12 year period, compared to over 200 ASCTs between 2006 and 2011, it is certainly not óstandard of careô as indicated in the BSBMT guidelines. This data is limited in providing any definitive conclusions about the role of allogeneic transplantation, although it is clear there is a small cohort who appear to have achieved long-term cure. Therefore the hypothesis that the outcome after allogeneic transplantation for patients with multiple myeloma justifies the increased toxicity by decreasing relapse is certainly supported in some cases, but is not universal and it remains difficult to know in whom that statement stands.

The FC RIC-SCT does have a very low NRM in comparison to most reported allogeneic studies. However, the most likely outcome remains relapse, with a higher NRM risk compared to ASCT. This data-set is too small to make recommendations regarding optimal patient selection. However, if patients with poor risk cytogenetics are able to overcome their adverse outcomes, then this is clearly a population in whom there is more justification to consider the allogeneic approach. Further studies with larger numbers of patients are required to help us make well-placed evidence-based decisions regarding allogeneic transplantation in this disease. Ongoing research is required to determine how best to keep NRM low whilst minimising post transplant relapse. In the interim, I would suggest that allogeneic transplantation is recommended in the BSBMT tables as a clinical option in first response and at relapse for both sibling and unrelated donors.

CHAPTER FOUR

Sequential transplantation in refractory and relapsed acute myeloid leukaemia and myelodysplasia

4.1 Introduction

The majority of patients with newly diagnosed acute myeloid leukaemia (AML) who receive intensive induction chemotherapy will achieve a complete remission.[126]

For those patients that achieve a remission following induction, 50% will relapse. Studies have demonstrated that whilst a second CR can be achieved with salvage chemotherapy, relapse follows soon after, with a median 3 year OS of between 8-29% and just over 10% achieving long term remission.[127-131] There is no standard of care with regards to salvage chemotherapy but allogeneic stem cell transplantation is recognised to be the most promising treatment strategy in this setting. [132, 133]

Approximately 20% of patients with AML will be refractory to induction therapy. Their prognosis is poor and salvage with chemotherapy alone is unlikely to result in a sustained remission. Again, allogeneic transplantation provides the best chance of prolonged survival in this setting.

The BSBMT recommend allogeneic transplantation in patients with refractory disease as a clinical option based upon studies that report an OS of approximately 30% (table 10). [134, 135] However, several studies have confirmed the importance of achieving a CR with salvage therapy before consolidating this response with an allogeneic transplant. Those patients not in remission at the time of transplant do worse and as such allogeneic transplantation in a second CR is classified as óSô by BSBMT (table 10).[136, 137]

Table 10: BSBMT Indications for SCT in AML (taken from BSBMT Indications for SCT Version Oct 13)

AML

		Sibling transplant	MUD transplant	Autograft	Comments
APL CR1		GNR	GNR	GNR	BCSH guidelines
APL CR2 PCR+		S	S	GNR	
APL CR2 PCR-		CO	GNR	S	
AML -good risk	**CR1**	GNR	GNR	GNR	BCSH guidelines AML15/16 trial protocols
	CR2	S	S	CO	
AML -standard risk	**CR1**	S	S	GNR	AML 15/16 protocols
	CR2	S	S	CO	
AML -poor risk*	**CR1**	S	S	GNR	AML 15/16 protocols
	CR2	S	S	CO	
AML not in remission		CO	CO	GNR	Fung et al [1], Cook et al [2]

* Poor risk defined as either 1. cytogenetics (MRC criteria), 2. Secondary or therapy – related AML, 3. Failure to achieve CR with standard AML induction therapy

The median age of diagnosis with AML is 65 years. The introduction of reduced-intensity conditioning has allowed allogeneic transplantation to be offered to patients who would not have been deemed fit enough for a myeloablative transplant by virtue of their age or performance status. RIC-SCT offers a significantly reduced toxicity profile compared to myeloablative conditioning. It relies on the graft-vs-leukaemia effect, rather than disease eradication through high dose chemotherapy and TBI.[37, 138]

There is evidence that conditioning intensity is important in controlling poor risk disease.[139] Therefore, there is concern that the reduced intensity approach may not be sufficient to hold aggressive disease such as primary refractory and relapsed AML whilst the GvL effect is allowed to occur. Furthermore, patients with poor risk disease are likely to have the most chemoresistant disease and the highest chance of relapse. They are consequently the most likely to struggle to achieve the remission status ideally required in order to proceed with allogeneic transplantation.

The sequential allogeneic transplantation approach was introduced to deal with these concerns by first giving intensive chemotherapy to reduce the leukaemic burden and then proceeding immediately with RIC-SCT without awaiting count recovery or confirmation of remission.[140]

Myelodysplastic syndrome (MDS) is another haematological disorder predominantly affecting the older population, with 80% of patients over the age of 60. It is recommended that patients with MDS who are high risk and under the age of 60 should have an allogeneic transplant (table 11).[141]

Table 11: BSBMT Indications for SCT in Myelodysplastic Syndrome (taken from BSBMT Indications for SCT Version Oct 13)

Indications for Transplantation for Adults with Myelodysplastic Syndromes

MDS

IPSS score	Autograft	Sibling Allograft	VUD allograft	UCBT
Low-Int-1	GNR	CO*	CO*	D**
Int-2, High	GNR	S	S	D**
t-MDS	GNR	S	S	D**

t-MDS: therapy related MDS

Reduced intensity conditioning protocols are recommended for patients aged 40-45 years or older, or in patients with pre-existing co-morbidities as defined using the HSCT co-morbidity index (HCT-CI)

*Allogeneic transplantation in patients with Low or Int-1 disease is generally considered at time of disease progression: progressive cytopenias and transfusion dependence, increasing blast counts, acquisition of adverse cytogenetic markers

**In view of the limited data on transplantation of adult patients with MDS using umbilical cord blood units, it is recommended that this should be performed within the confines of a clinical research protocol

There is debate about the role and type of treatment prior to transplant. As with AML, there is some evidence from retrospective studies suggesting that achieving a CR prior to transplant results in better outcomes.[142] However, using AML-like induction chemotherapy to achieve this can be associated with delayed haematological recovery and treatment related toxicities that may actually make transplantation a less viable option. Again, the sequential approach allows these concerns to be bypassed by reducing disease bulk before proceeding directly to transplant.

Since Schmid et al reported this approach in 2005 there have been several reports concurring that this strategy is effective (table 12).[143-151] However, all groups have used T cell depletion with ATG as part of their conditioning and the majority have used TBI. The toxicity associated with TBI means that its use is potentially limited in older patients and those with comorbidities.[152]

There is a clear rationale behind the sequential approach. However, given that the implicated patient population is older and more likely to have a higher HCT-CI, we performed a prospective single-centre study using the sequential approach with fludarabine and cyclophosphamide T-replete conditioning. This is a reduced intensity approach associated with low NRM in the non-sequential setting and therefore has the potential to widen the cohort of patients that may benefit from allogeneic transplantation in this setting.[111, 153-156]

4.2 Hypothesis

The sequential transplantation approach increases the probability of getting a patient to transplant and should be considered as standard of care for patients with relapsed and refractory AML and high risk myelodysplasia.

4.3 Methods

4.3.1 Patient eligibility

Fourty-four patients were enrolled into the study. Patients with refractory or relapsed AML and high-risk MDS were all eligible. Patients had to have an identified donor at the time of entry into the study or have disease stable enough to allow a donor search to occur before commencing treatment. Refractory AML was defined as the presence of disease following induction chemotherapy (morphological evidence of >5% blasts on bone marrow) or relapse within 6 months of CR1. Four of the enrolled patients did not undergo transplantation; 2 with relapsed AML had an identified matched unrelated donor who was deemed unfit to donate by the donor registry and no other suitable donors could be identified within a suitable time-

		Schmid et al[140]	Liu et al[143]	Saure et al[144]	Chemnitz et al[145]	Schneidawind et al[146]
Study Type		Prospective	Prospective	Prospective	Prospective	Retrospective
N		75	51	30	17	62
Median F/U (m)		31.5	41	28	12	17
Med age (range) yrs		52.3 (18-65)	30 (14-53)	49 (36-66)	57.4 (40-69)	55 (20-72)
HCT-CI	**Low (%)**	NA	NA	40	6	NA
	Med/ High (%)			60	94	
Diagnosis	**Poor risk AML CR1 (%)**	10.6	0	0	29	0
	AML CR2 (%)	10.6	0	0	7	0
	Relapsed AML (%)	37.3	19.6 (AML/ALL)	0	35	42
	Refractory AML (%)	28	76.5 (AML/ALL)	0	29	58
	High Risk MDS CR1 (%)	0	0	0	0	0
	Untreated AML/ HR MDS(%)	0/13.3	0	66/33	0	0
	MPS(%)	0	0	0	0	0
Cytogenetics	**Favourable(%)**	4	7.8	40	29	0
	Intermediate(%)	42	35	23		66
	Poor(%)	49	39	37		33
Donor	**Matched MUD (%)**	40	24	44	47	35
	Matched SIB (%)	41.3	44	43	24	18
	MM MUD/SIB (%)	18.7	32	13	29	46
Chemotherapy		FLAMSA	flu/ AraC/Etop	FLAMSA	FLAMSA	FLAMSA
Conditioning		4Gy	4.5Gy			
		Cyclo 40/60mg/m^2 x2	Cyclo 60mg/m^2 x2	Melphalan 100-200mg/m^2	Treosulfan 10g/m^2 x3	Flu/Bu/ATG
		ATG 10-20mg/kg x3	Etop 600mg/d x2	Thiotepa 10mg/m^{2}x2 <55yrs	Cyclo 40/60mg/m^2 x 2	Bu/Cy/ATG
			ATG if VUD/MM SIB	ATG 10-20mg/kg x3 (VUD)	ATG 10-20mg/kg x3	TBI(4Gy)/Cy/ATG
Neut Engraft (d)		14	12	13	18	17
CR D30 (%)		88	94	97	94	NA
aGVHD(%) {>Gr II)		45	32	73 (I-IV) 20 (III-IV)	20	21
cGVHD	**Limited (%)**	26	29	56	43	NA
	Extensive(%)	19	21	20	14	26
OS	**1yr (%)**	NA	57	NA	62	NA
	2 yr (%)	42	NA	70	NA	39
	3yr (%)	NA	48	NA	NA	NA
DFS	**1yr (%)**	NA	48	NA	55	NA
	2yr (%)	40	NA	63	NA	26
	3yr (%)	NA	42	NA	NA	NA
NRM	**<30d (%)**	7	3.9	NA	5.8	NA
	100d(%)	20	19	NA	12	NA
	1yr(%)	33	NA	30	20	NA
	2yr(%)	NA	NA	NA	NA	22

Table 12: Reported sequential transplantation studies in acute leukaemia and MDS

NA Not available; FLAMSA fludarabine 30mg/m^2, cytarabine 2g/m^2, amsacrine 100mg/m^2 days D-12 to D-9

		Detrait et al[149]	Cluzeau et al[150]	Krejci et al[147]	Buchholz et al[148]	Michallet et al[151]
Study Type		Retrospective	Retrospective	Prospective	Retrospective	prospective
N		40	17	60	27	26
Median F/U (m)		6	14	37	35.9	na
Med age (range) yrs		52 (32-66)	53 (32-64)	52 (20-63)	58 (19-69)	55 (24-67)
HCT-CI	**Low/ med & high (%)**	na	59/41	42/58	na	na
Diagnosis	**Poor risk AML CR1 (%)**	10	0	16.7	14.8	100
	AML CR2 (%)	0	0	25	3.7	0
	Relapsed AML (%)	62.6	65	18.3	0	0
	Refractory AML (%)	10	35	40	55	0
	High Risk MDS CR1 (%)	17.5	0	0	3.7	0
	Untreated AML/ HR MDS(%)	0	0	0	0/18.5	0
	MPS(%)	0	0	0	3.7	0
Cytogenetics	**favourable(%)**	0	0	10	0	0
	Intermediate(%)	30	59	62	78	15
	Poor(%)	52.5	41	17	22	85
Donor	**Matched MUD (%)**	35	48	48	63	31
	Matched SIB (%)	45	35	25	22	35
	MM MUD/SIB (%)	20	17	27	15	34
Chemotherapy		FLAMSA	Thio200mg/m^2 x3	FLAMSA	Clof 30mg/m^2 x5	FLAMSA
			Etop 200mg/m^2 x3		Arac 1g/m^2 x5	
			6MP75mg/m^2 x14		5 rest days	
Conditioning		4Gy or BU	Flu 30mg/m^2 x4	4Gy	4Gy	4Gy or BU
		Cyclo 40/60mg/m^2 x2	Bu 3.2mg/kg x2	Cyclo 40/60mg/m^2 x2	Cyclo 40/60mg/m2 x2	Cyclo 40/60mg/m^2 x2
		ATG 5mg/kg x3	ATG 2.5mg/kg x2	ATG 10-20mg/kg x3	ATG 4.5-7.5mg/kg x3	ATG 5mg/kg x3
Neut Engraft (d)		NA	NA	17	16	NA
CR D30 (%)		NA	86.7	78	89	NA
aGVHD(%) {>Gr II)		25	41	47 (1-IV)	52% (1-2 only)	21.7
cGVHD	**Limited /Extensive(%)**	NA	0/7	39/16	21/8	17.4/4.3
OS	**1yr (%)**	30	29.4	45	NA	NA
	2 yr (%)	NA	NA	NA	56	58
	3yr (%)	NA	NA	42	NA	NA
DFS	**1yr (%)**	NA	17.7	38	NA	NA
	2yr (%)	29	NA	NA	50	NA
	3yr (%)	NA	NA	33	NA	NA
NRM	**<30d (%)**	NA	11.7	15	11	NA
	100d(%)	14	NA	NA	15	NA
	1yr(%)	22	NA	25	NA	NA
	2yr(%)	NA	NA	NA	35	13
	3yr(%)	NA	NA	28	NA	NA

Table 12(cont): Reported sequential transplantation studies in acute leukaemia and MDS NA Not available frame. Two other patients died before they were able to proceed with transplant (one with MDS died from infection and the other from relapsed AML). Consequently, 40 patients were transplanted between September 2007 and February 2014. The hospital ethics committee approved the protocol and written informed consent was obtained from all patients and donors in accordance with the Declaration of Helsinki (appendices 1& 2).

4.3.2 Treatment protocol

Patients received daunorubicin 45mg/m^2 from D-15 to D-13 and cytarabine 1500mg/m^2 *b.d* from D-15 to D-9. After 2 rest days, RIC consisted of fludarabine 25mg/m^2 on D-6 to D-2 and cyclophosphamide 1g/m^2 on D-3 and D-2. GvHD prophylaxis was with methotrexate (5 mg/m^2 on days +1, +3 and +6), and ciclosporin (CSA), 5 mg/kg on day -2 and 3 mg/kg per day thereafter. Trough CSA levels were measured in whole blood twice weekly post-transplant. CSA was dosed to maintain levels between 150ï 300mcg/L. Tapering was commenced at day +90. CSA taper was commenced earlier in those patients with mixed donor chimerism. DLI would be considered 30 days after discontinuation of immunosuppression in the presence of mixed chimerism or evidence of persistent disease.

4.3.3 Supportive care

All patients received antifungal and antimicrobial prophylaxis with fluconazole and ciprofloxacin until neutrophil engraftment. All patients were commenced on cotrimoxazole following engraftment as prophylaxis against *Pneumocystis jiroveci*. All patients received antiviral prophylaxis with aciclovir for a minimum of 6 months or longer if still on immunosuppression. Peripheral blood samples were monitored weekly for CMV re-activation by quantitative polymerase chain reaction (PCR). Patients with evidence of CMV re-activation were treated with pre-emptive valganciclovir.

4.3.4 Patient evaluation

Neutrophil recovery was defined as time to a count of 0.5×10^9/L for 2 consecutive days. Chimerism was measured on whole peripheral blood samples by PCR analysis of variable number tandem repeat polymorphisms at days +30, +60, +90 and thereafter as appropriate. T lineage-specific chimerism was performed after immunomagnetic sorting of CD3$^+$ T cells.

Acute GVHD was diagnosed and graded using current consensus criteria.[113] Chronic GvHD was diagnosed and scored using Seattle criteria.[114] GvHD was managed by institutional protocol. Disease relapse was defined as detectable disease by morphology or cytogenetics. Patients were deemed to have persistent disease if detectable by bone marrow morphology assessed at day+30 and were censored at this date for relapse. The median follow-up of surviving patients was 51 months. Patients who died before day+30 were excluded from engraftment and chimerism analysis. NRM was defined as death in the absence of disease relapse or persistence.

4.3.5 Statistical analysis

Survival curves were constructed using the method of Kaplan and Meier and differences between groups were assessed using the log-rank statistic. Patients were censored at last follow-up when calculating overall survival. Univariate analysis of association of outcomes with risk factors was performed. These included age (60 yrs or Ó60 yrs), diagnosis, cytogenetics (adverse or intermediate), donor type, sex mismatch, major ABO mismatch, $CD34^+$ and $CD3^+$ cell doses, CMV risk, peripheral blood chimerism at day+30 and the presence of acute and chronic GVHD. *P*-values of less than 0.05 were considered statistically significant. Data were analyzed using SPSS v22 (IBM, New York). Cumulative incidence and multivariate analysis was performed using STATA.

4.4 Results

4.4.1 Patient characteristics

Patient, donor and graft characteristics are summarized in table 13. Forty patients underwent sequential transplantation. The aggressive nature of acute myeloid leukaemia and the time-scale involved in identifying donors meant that many patients with relapsed and refractory AML who may have been eligible for the study were not enrolled because a donor was not identified prior to commencing therapy. The median age of the patient cohort was 53 years (range 23-68 years). Eight patients (20%) were aged 60 years or over. Thirty-four patients (85%) had AML. 50% of AML patients were refractory, 30% had relapsed disease and 5% were untreated with bone marrow blasts between 20-30%. Patients with MDS made up 15% of the total cohort. Seven patients (17.5%) had undergone previous allogeneic transplantation. There were no patients with favourable risk cytogenetics. The proportions of those with intermediate and high risk are shown in table 13. Four patients had monosomal karyotypes. This was too small a group to perform separate analyses on. Whilst those treated in the latter stages of the study had FLT3 and NPM results available, the majority of patients were enrolled into the study before molecular studies had become routine diagnostic tests and therefore this data was not available for analysis. According to the HCT-CI score, 35%, 30% and 35% of patients were classified as low, intermediate and high respectively.[54]

Table 13: Patient characteristics in sequential allograft study

			N	%
			40	
Gender	**F**		13	32.5
	M		27	67.5
Age	**Median (range)**		53 (23-68)	
	≥60yrs		8	20
Diagnosis	**Refractory AML**	**Total**	20	50
		Int CG	13	
		Poor CG	7	
	Relapsed AML	**Total**	12	30
		Int CG	10	
		Poor CG	2	
	Untreated AML	**Total**	2	5
		Int CG	1	
		Poor CG	1	
	Myelodysplasia	**Total**	6	15
		IPSS low	0	
		IPSS INT1	1	
		IPSS INT2	5	
		IPSS High	0	
HCT-CI	**Low**		14	35
	Intermediate		12	30
	High		14	35
Donor	**Matched Sibling**		19	47.5
	Matched VUD		20	50
	Mismatched VUD		1	2.5
ABO mismatch	**Yes**		12	30
	No		28	70
Gender mismatch	**Female donor/male recipient**		8	20
	Any other combination		32	80
CMV mismatch	**Yes**		28	70
	No		12	30
Prev transplant	**Yes**		7	17.5
	No		33	82.5

CG cytogenetics; IPSS International prognostic scoring system

Patients were transplanted using HLA-matched related donors (n=19, 47.5%) or volunteer unrelated donors (n=21 52.5%). One VUD was a 1C single antigen mismatch. There was a major ABO mismatch in 12 cases (30%). In 70% of cases, there was a CMV reactivation risk. There were 9 cases of a male recipient and female donor (19.6%). Median donor age was 41 years (range 22-68 years). Given the median age of patients was 53 yrs, it was not surprising that there were significantly more sibling donors above the median donor age compared to matched unrelated donors; (p=0.01). However, stem cell graft characteristics were not significantly different between related and unrelated donors.

Intermediate resolution HLA-typing was used for Class I (HLA-A and HLA-B, HLA-C) and high resolution molecular typing for Class II (DRB1 and DQB1) alleles. Unrelated donors were fully matched for HLA-A, -B, -C, -DRB1, and DQB1. Stem cell source was granulocyte colony-stimulating factor-mobilized peripheral blood stem cells (PBSC) (n=39) or fresh unmanipulated bone marrow (n=1). Patients received a median $CD34^+$ cell dose of 5.88 x 10^6/kg (range 1.76-26.20) and median $CD3^+$ T cell dose of 3.31 x 10^8/kg (range 0.36-13.37).

4.4.2 Engraftment and chimerism

Six patients died in the cytopenic phase. Excluding those, all other patients engrafted and median time to neutrophil engraftment was 20.5 days (range 11-29). FDC was achieved in 78% of all assessable patients. Of those, 56% of patients had reached FDC by D30. Full T-cell chimerism (FTCC) was achieved in 81% of all evaluable patients. Of these, 64% achieved FTCC by D30. No patients received DLI post transplant.

4.4.3 Toxicity and non-relapse mortality

Median duration of hospital stay was 38 days (range 30-127). Cumulative incidence of NRM with competing risks was 38%. Of the 6 patients who died in pre-engraftment, 5 were secondary to neutropenic sepsis (the remainder was secondary to refractory disease). Patient age had no statistical impact on overall NRM, although 50% (n=3) of deaths before D30 were in patients over 60. 41% of all NRM deaths were secondary to infection, 17.6% were due to acute GVHD and 23.5% were secondary to chronic GVHD. 17.6% were due to other causes including pneumonitis, subdural haemorrhage and liver failure. Nine patients had CMV reactivation; this included 1 patient with CMV colitis but there was no other CMV related disease. No other significant viral infections occurred.

4.4.4 Graft vs host disease

12 patients (35% of assessable patients) developed acute GVHD (grades II-IV) of whom 25% (n=3) died from GVHD. Median time to acute GVHD was 38 days (range 24-97 days). Nine patients (33% of all assessable patients) had chronic extensive GVHD or GVHD on tailing ciclosporin and 44% of these died from GVHD or infection in the context of immunosuppression for GVHD. Median time to chronic GVHD was 159 days (range 133-371).

4.4.5 Relapse and survival outcomes

Median survival of all patients was 9 months. 1 year and 3 year OS was 42% and 30% respectively. Median PFS was 8 months. There were clear survival trends depending on diagnosis (figure 15). When patients with MDS and relapsed AML were compared to those with refractory AML, median OS was 25 vs 7 months respectively; p=0.029 (figure 16). Univariate analysis demonstrated that no other factors impacted upon OS. The impact of diagnosis on PFS was less marked (figure 17).

Figure 15: OS using sequential allogeneic transplantation by diagnosis

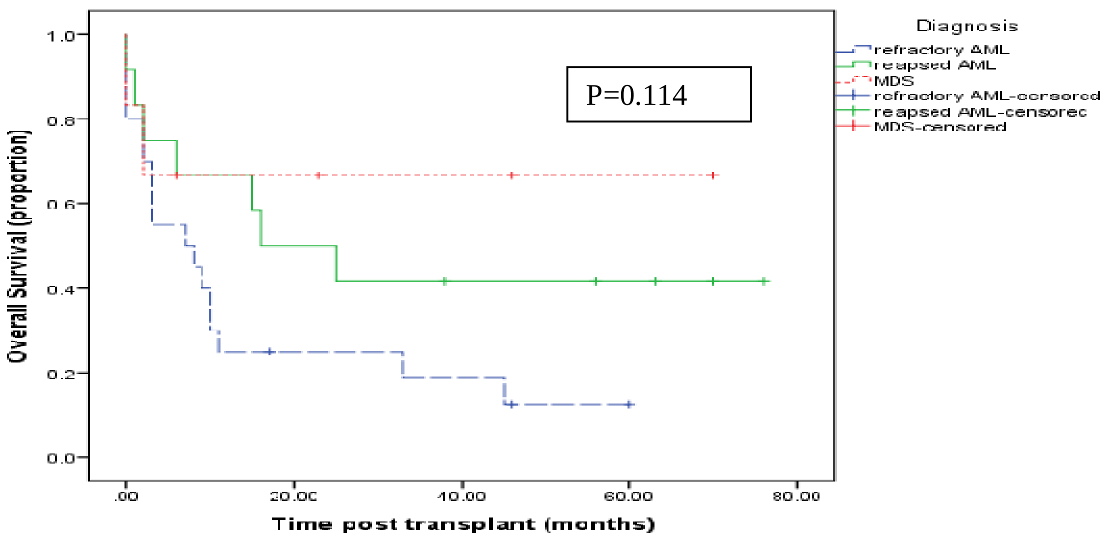


Figure 16: OS using sequential allogeneic transplantation comparing refractory AML to relapsed AML and MDS

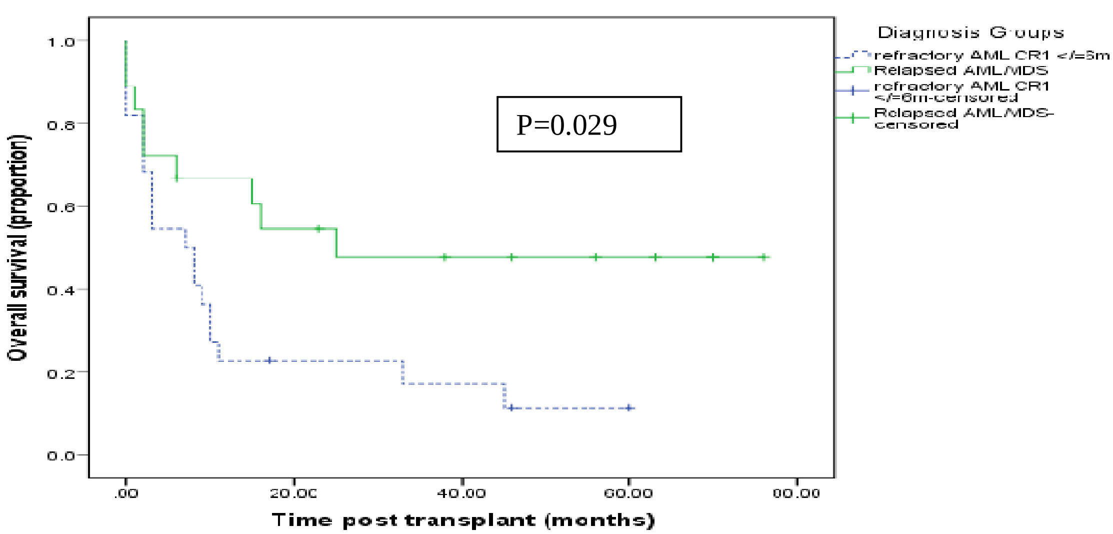
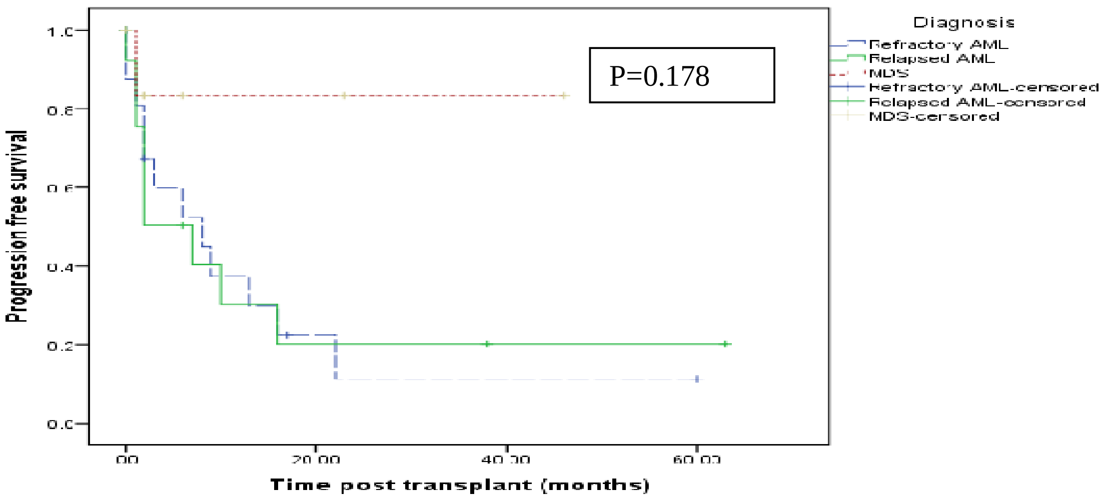


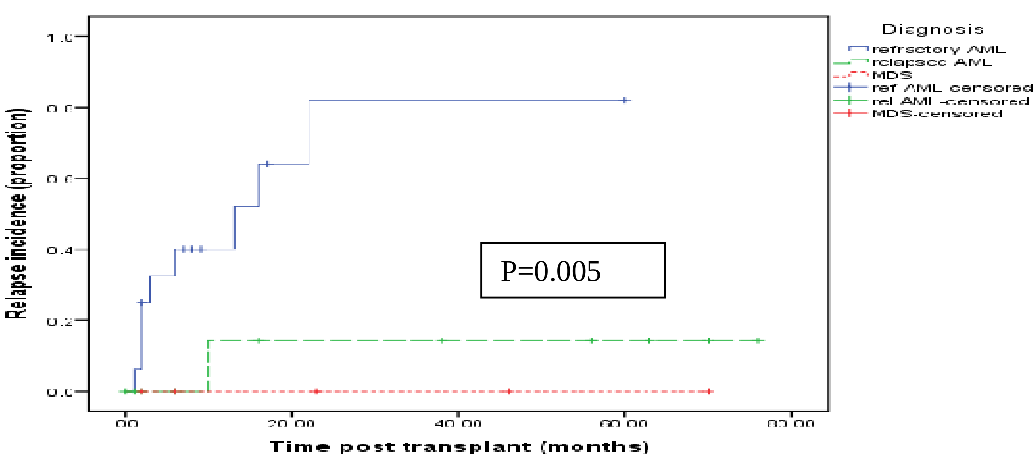
Figure 17: PFS using sequential allogeneic transplantation by diagnosis



89.7% of all assessable patients had no evidence of disease on their D30 bone marrow. Three patients had refractory disease on their D30 bone marrow. A further 9 patients relapsed post transplant. Median time to relapse in those not refractory at D30 was 211 days (range 76-671). Cumulative incidence of refractory or relapsed disease was 35%.

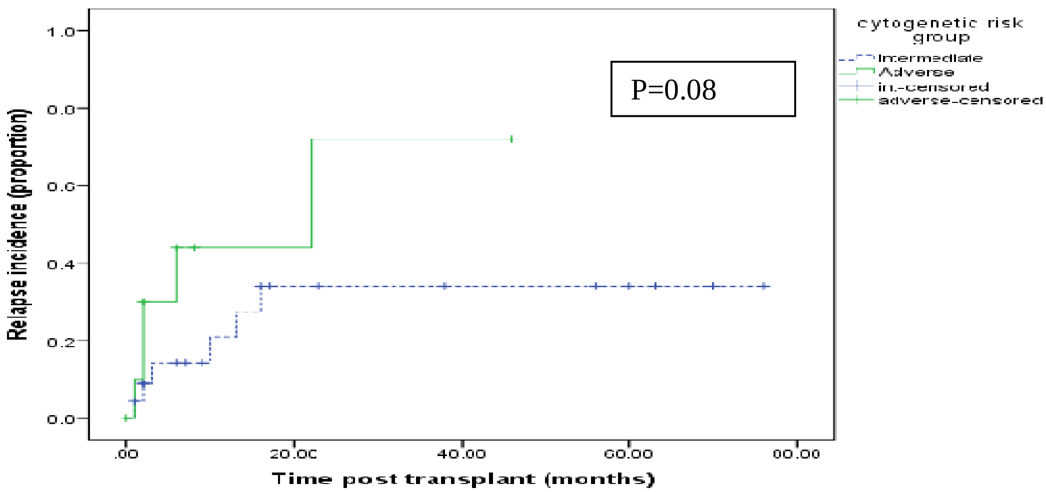
Diagnosis had a significant impact on risk of relapse; p=0.005 (figure 18). No patients with MDS relapsed. Only one patient with previously relapsed AML relapsed again post transplant with all other relapses occurring in the refractory AML cohort.

Figure 18: Relapse using sequential allogeneic transplantation by diagnosis



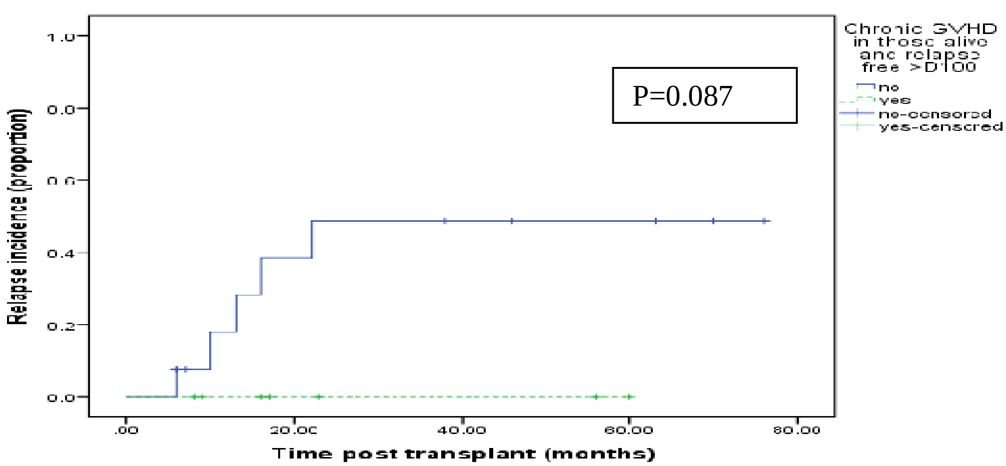
The presence of poor risk cytogenetics also trended towards a statistical impact on relapse, p=0.08 (figure 19). There were no patients with good risk cytogenetics in this cohort. Median time to relapse was not reached in those with intermediate risk cytogenetics, but was 212 days in those with adverse risk. The number of MDS patients was too small to separately evaluate the impact of MDS prognostic scoring.

Figure 19: Relapse using sequential allogeneic transplantation by cytogenetic risk in AML and MDS



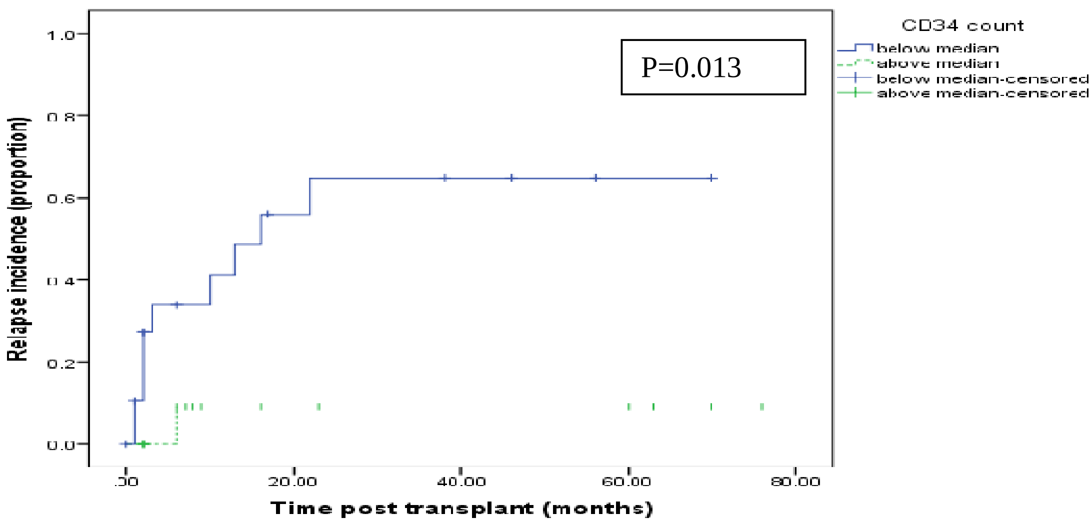
The presence of chronic GVHD demonstrated a striking disparity on the Kaplan-Meier graph with no patients who developed chronic GVHD relapsing. However, only a trend towards statistical significance was seen; p=0.087 (figure 20), most likely due to small patient numbers eligible for analysis beyond D100. Acute GVHD had no impact on PFS or OS.

Figure 20: Relapse using sequential allogeneic transplantation by presence of chronic GVHD



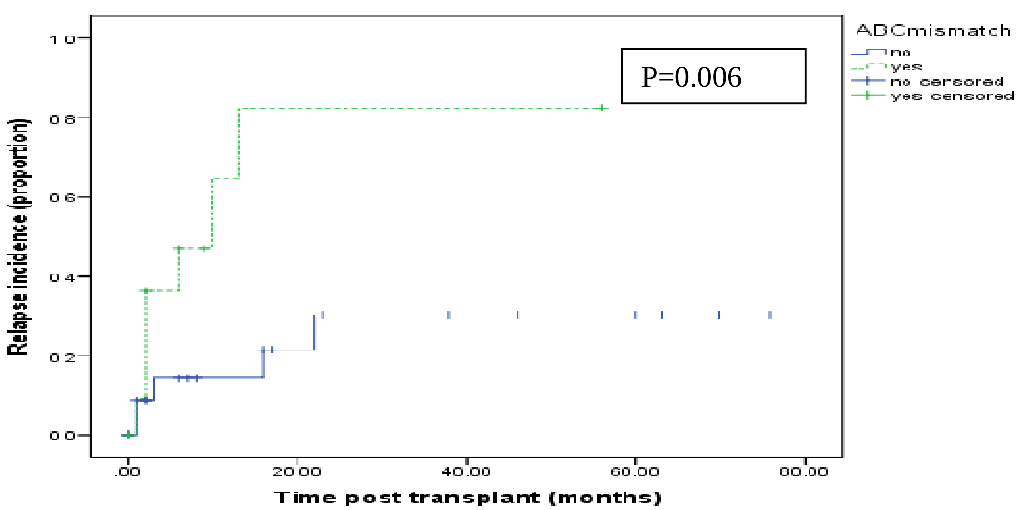
A higher CD34 count (above the median of 5.88×10^6) was associated with a significant reduction in relapse; p=0.013 (figure 21).

Figure 21: Relapse using sequential allogeneic transplantation by CD34 count above and below median



The presence of a major ABO mismatch was surprisingly associated with an increased incidence of relapse, p=0.006 (figure 22). There was a similar trend towards significance for PFS (0.065) but there was no effect on OS.

Figure 22: Relapse using sequential allogeneic transplantation by presence of major ABO mismatch



4.5 Discussion

This study evaluated the outcomes of 40 patients with relapsed or refractory AML and high risk myelodysplasia using a previously unreported sequential approach with intensive chemotherapy comprising daunorubicin and cytarabine followed by a reduced-intensity T replete fludarabine cyclophosphamide allogeneic transplant protocol.

It has been demonstrated that patients with MDS or AML who undergo allogeneic transplantation with active disease have worse outcomes compared to equally matched patients in CR.[157] The sequential approach was devised with the aim of reducing the burden of disease in order for the GvL effect to have sufficient opportunity to occur in those with high risk disease.[140]

This is the only study to report this approach with no T cell depletion, even in the context of unrelated donors. This T cell replete strategy is an extension of the fludarabine cyclophosphamide conditioning approach used at St Bartholomew's for fully matched allogeneic transplants with a very favourable toxicity profile reported.[111, 155, 156] It offers a very low incidence of post transplant viral complications and a low demand of DLI.

Despite the sequential strategy, four enrolled patients did not get to transplant highlighting the difficulties of successfully proceeding with SCT in this high-risk cohort.

Cumulative incidence NRM was clearly significant at 38%. The range reported in the literature varies between 22-35%.[140, 144, 145, 147, 148] Cause of NRM was split equally between infection and GVHD. The rationale behind T cell depletion is to minimise GVHD and its associated toxicities. However the incidence of both acute and chronic GVHD at 35% and 33% respectively was lower than several T deplete studies (aGVHD 18-73%, cGVHD 7-76%). The high NRM may be accounted for by the characteristics of the patients included in this study. Their median age was 53 years which was comparable to most (although not all) studies. 65% of patients had a HCT-CI of medium or high. Most sequential studies made no comment regarding HCT-CI, but two reported proportions of medium and high HCT-CI between 12-30% and only 3 had a comparable proportion to our own cohort.[144, 145, 147, 150]

One-year OS was within the range reported by previous studies at 42% (29-62%). 3 year OS was 30%, under the reported range (42-49%). It is important to note that the median follow up of 51 months was significantly longer than any other reported study and most had not yet reached 3-year follow-up. These inferior survival outcomes may well be explained by the patient cohort, which had a particularly poor disease characteristic profile. Unlike other reported sequential studies, the patient cohort in this study included no patients in CR and no patients with good risk cytogenetics, both of whom are reported to have better outcomes.[131, 136] Furthermore, the proportion of patients with myelodysplasia was much higher in one study and given the superior outcomes seen in MDS patients in this study, this may well contribute to their improved survival outcomes.[144] The only reported study comparable to this one in terms of age, HCT-CI and patient disease status had a short follow up at 14 months but interestingly reported the worst 1 year OS at 29% and a D30 NRM of 11.7%.

Breems et al demonstrated that there are factors that help to identify which patients with relapsed AML may benefit most from transplant.[131] According to their prognostic scoring system, which includes age, previous transplant, cytogenetic risk and length of CR1, all of the relapsed AML patients in this study fell into the worse two prognostic categories with a predicted 5 year OS of 4-18%. The OS of patients with relapsed AML in this study was 41.7% at 5 years and therefore significantly better than their predicted outcome outside of the sequential setting.

There were only six patients with MDS in this study which makes drawing any robust conclusions about this group difficult. According to the WHO classification based prognostic scoring system (WPSS), all of the patients in this study had a score of high or very high. This gives them a predicted median survival of between 12 and 21 months.[158] If the MDS patients in this study are looked at alone, there are no relapses and their median OS is not reached, with 67% OS at 5 years. Perhaps, larger patient numbers would change this data. However, it suggests that the sequential strategy might be particularly effective for patients with MDS. This is supported by data from Saure et al.[144] Their OS data is the best of all reported sequential studies (2 year OS of 70%) and their cohort predominantly includes patients with MDS.[144]

Patients who benefited less well from this approach were those with refractory AML and those with adverse cytogenetics. With an OS of less than 20% in both of these groups, it appears there is little to be gained from this approach compared to standard and possibly less toxic alternatives.

This data clearly demonstrated the graft-vs leukaemia effect. Those patients who developed chronic GVHD, and did not succumb to its toxicities, did not relapse with an OS of 50% in this subgroup of patients. Therefore it is clear, that conditioning that aims to minimise GVHD, is also minimising the potential benefits of GvL. Work continues to try and pull apart GVHD and GVL. In the interim, the aim, although challenging, should be to select patients who are best able to tolerate the potential associated GVHD toxicities. 75% of the GVHD-related deaths were in patients above the median age of 51 years. Furthermore, of the 6 observed deaths in aplasia, 3 were in patients >60 years. These deaths are clearly multifactorial, and age is only one factor, but it suggests that this treatment strategy and its associated toxicities are perhaps tolerated less well by the older population.

A higher CD34 count was associated with a reduction in relapse. This has been demonstrated in other studies with larger patient numbers, outside of the sequential setting and furthermore that association has been reported to be most marked in high-risk diseases where there is more evidence of the GVL effect.[159-161] This has been postulated to be immune-mediated, possibly linked to a natural killer(NK) cell mediated graft-vs-leukaemia effect in view of the fact that the majority of early peripheral blood lymphocytes post transplant are NK cells.[162] The lack of OS benefit seen with an increased CD34 count may be due to the occurrence of increased GVHD, which has previously been reported to be associated with higher CD34 counts.[163] In this analysis, the median CD34 count in those who had GVHD-related deaths was 8.3 $x10^6$ (range 4.6-26.2) compared to a lower median CD34 count of 4.4 (range 1.95-16.06) in those who had disease related deaths.

The observation that the presence of a major ABO mismatch impacted adversely on relapse risk is interesting. Whilst there have been reports associating ABO mismatch with reduced survival, possibly because of delayed engraftment and the consequent associated risks, no association with increased disease relapse has been reported. Whilst, the sample size must be remembered, the effect is striking. I would hypothesise that the ABO mismatch allows pre-formed antibodies to cross-react with antigens on the donor cells. This leads to cytokine activation and perhaps results in subsequent damage to donor cells and subsequently less effective GvL. As a result of the impaired GvL effect, relapse is more likely.

The rationale of using lower intensity chemotherapy and conditioning facilitated the delivery of treatment and transplantation to an older, less fit patient group, whilst maintaining comparable outcomes. The sequential approach has the potential to avoid the toxicities associated with induction chemotherapy, which are less likely to work in patients with poor-risk disease and ultimately may result in a failure to proceed with transplantation. There is also a potential financial and resource gain associated with avoiding potentially 2 or 3 cycles of intensive chemotherapy before a ‘standard’ allogeneic transplant.

NRM is high and OS is low compared to many other allogeneic transplant scenarios, but this is a cohort of patients with an otherwise dismal prognosis. This study has provided clear identification of those patients who are most likely to benefit; namely those with MDS and relapsed AML. Their outcomes were far superior to those predicted using conventional treatments. I would recommend that patients with adverse cytogenetics and refractory AML have little to gain from this approach and perhaps less toxic, non-curative therapies or clinical trials are more appropriate. The significant early mortality in those over 60 suggests that perhaps this should approach be reserved for younger patients, despite the ôlighterô conditioning.

Without a randomised prospective study comparing the sequential approach to conventional allogeneic transplantation, there is insufficient evidence to consider it standard of care and therefore the hypothesis in this study is rejected. However, there have been several studies using the sequential approach, all demonstrating acceptable outcomes in scenarios where prognosis is exceptionally poor. This study supports those findings and I would therefore suggest that the BSBMT makes an addition to its current recommendations such that the sequential approach should be considered in patients with relapsed AML and myelodysplasia as a clinical option.

CHAPTER FIVE

Allogeneic Stem Cell Transplantation in Lymphoma

5.1 Introduction

For both indolent lymphomas such as follicular lymphoma (FL) and high grade lymphomas such as Diffuse Large B Cell Lymphoma (DLBCL) and Hodgkin lymphoma (HL), autologous transplantation is the standard of care in those with primary resistant or relapsed disease (tables 14, 15 and 16).[164, 165]

Table 14: BSBMT Indications for SCT in Follicular Lymphoma (taken from BSBMT Indications for SCT Version Oct 13)

Follicular Lymphoma

	Autograft	Sibling transplant	MUD transplant
CR1/PR1	GNR[1]	GNR	GNR
CR/PR>1	S[2]	CO[3]	CO[3]
Chemorefractory (<PR)	GNR	D	D
Primary resistant* - **Sensitive to salvage** - **Resistant**	 CO[2] GNR	 CO[3] GNR	 CO[3] GNR
Relapse post autograft	GNR	S[4]	S[4]

* to multi-drug regimen; note, in transformed disease transplantation should be considered standard in those sensitive to salvage

References

1. Lenz et al Blood 2004; 104: 2667-2674, Sebban et al Blood 2006; 108: 2540-2544, Gyan et al Blood 2009; 113: 995-1001, Montoto et al Haematologica 2013; 98: 1014-1021
2. Schouten et al JCO 2003; 21: 3918-3927, Montoto et al Haematologica 2013; 98: 1014-1021, Villa et al JCO 2013; 31: 1164-1171, Eide et al BJH 2011; 152: 600-610, Williams et al JCO 2001; 19: 727-735, Ban-Hoefen et al Leuk Lymphoma 2012; 53: 830-835
3. Patients considered at high risk of early failure following an autograft i.e. should be considered in young patients with short 1st response (<12-18 months) or high FLIPI at relapse or failure to achieve CR with salvage. van Besien et al Blood 1998; 92: 1832-1836, Morris et al Blood 2004; 104: 3865-3871, Robinson et al Blood 2002; 100: 4310-4316, Faulkner et al Blood 2004; 103; 428-434, Thomson et al JCO 2010; 28: 3695-700, Robinson et al BMT 2013; doi: 10.1038/bmt.2013.83
4. Allogeneic transplantation should be considered standard in chemosensitive patients if the predicted NRM is acceptable. Morris et al Blood 2004; 104: 3865-387, Robinson et al Blood 2002; 100: 4310-4316, Thomson et al JCO 2010; 28: 3695-700, Montoto et al Haematologica 2013; 98: 1014-1021, Faulkner et al Blood 2004; 428-434, Rezvani et al JCO 2008; 26: 211-217

Table 15: BSBMT Indications for SCT in DLBCL (taken from BSBMT Indications for SCT Version Oct 13)

DLBCL

	Autograft	Sibling transplant	MUD transplant
CR1	GNR[1]	GNR	GNR
CR/PR>1	S[2]	CO[3]	CO[3]
Chemorefractory (<PR)	GNR	GNR	GNR
Primary resistant - **Sensitive to salvage** - **Resistant**	 S[4] GNR	 S[4] GNR	 S[4] GNR
Relapse post autograft	GNR	S[5]	S[5]

References

1. Cochrane database
2. Philip et al NEJM 1995; 333: 1540-1545, Moore et al. BJH 2012; 156: 142-143
3. Chopra et al JCO 1992; 10: 1690-1695, Bierman et al JCO 2003; 21: 3744-3753, Bacher et al Blood 2012; 120: 4256-4262
4. Consolidation of response with a high dose transplant procedure should be considered standard practice in those patients failing to achieve CR with first line treatment who remain chemosensitive to salvage therapy; choice of modality will depend upon predicted NRM, & depth of response to salvage. Bacher et al Blood 2012; 120: 4256-4262, Lazarus et al Biol Blood Marrow Transplant. 2010; 16: 35-45
5. Allogeneic transplantation should be considered standard in chemosensitive patients if the predicted NRM is acceptable. Morris et al Blood 2004; 104: 3865-387, Sirvent et al Biol Blood Marrow Transplant. 2010, 16: 78-85, Rezvani et al 2008 BJH; 143: 395-403, van Kampen et al JCO 2011; 29: 1342-1348, Rigacci et al Ann Hematol. 2012; 91: 931-993

Table 16: BSBMT Indications for SCT in Hodgkin Lymphoma (taken from BSBMT Indications for SCT Version Oct 13)

Hodgkin Lymphoma

	Autograft	Sibling transplant	MUD transplant
CR1	GNR	GNR	GNR
CR/PR>1	S[1]	CO[2]	CO[2]
Chemorefractory (<PR)			
- **MR/stable**	GNR	CO[2]	CO[2]
- **Progressive**	GNR	D	D
Primary resistant			
- **Sensitive to salvage**	S[1]	CO[2]	CO[2]
- **Progressive**	GNR	GNR	GNR
Relapse post autograft	CO[3]	S[4]	S[4]

References

1. Linch et al Lancet 1993; 341: 1050-1054, Schmitz et al Lancet 2002; 359: 2065-2071; BCSH guideline 2013
2. Patients considered at high risk of failing an autograft e.g. PET⁺ post salvage, less than PR post salvage, multiple lines to achieve CR; Thomson et al Leukemia 2013; 27:1419-22; current NCRN trial (Peggs et al); BCSH guideline 2013
3. Should be considered a clinical option with very late relapse e.g. > 5 years. Thomson et al Leuk Lymphoma 2007; 48: 881-884; BCSH guideline 2013

The introduction of RIC-SCT in the 1990s has resulted in a rise in the use of this treatment modality in virtually all lymphoma subtypes, following the unacceptable NRM that was reported with myeloablative conditioning.[166-169] A graft-vs-lymphoma effect has been convincingly demonstrated by virtue of disease responses following withdrawal of immunosuppression, administration of DLI and the observation in some reports that chronic GVHD is associated with a reduced relapse rate.[170-176] This has resulted in RIC-SCT offering a potentially curative role in many lymphoma subtypes, and particularly in subgroups that face an otherwise poor prognosis.

However, whilst the NRM is reduced compared to MA conditioning, it remains significant and therefore its role in terms of patient selection and timing for most subtypes remains controversial. Allogeneic transplant is considered standard of care in those who have relapsed following ASCT (tables 14, 15 & 16). However, there is an increasingly grey area over the role of allogeneic transplant at first relapse and whether there are scenarios in which it should be considered in place of an ASCT following salvage chemotherapy. Furthermore, in subtypes such as Mantle Cell Lymphoma (MCL) and Peripheral T Cell lymphoma (PTCL), the role of allogeneic versus ASCT as consolidation in first response and at relapse is another area of debate with the BSBMT recommending it as a ‘clinical option’ in both scenarios (tables 17 and 18).

This chapter is a review of RIC-SCT performed in patients with lymphoproliferative disorders (LPD) between 2005 and 2013 at St Bartholomew’s Hospital. Patients with chronic lymphocytic leukaemia (CLL) were excluded from this report as they are subject to a separate analysis outside of this thesis. This retrospective analysis aimed to assess the outcomes of these patients and to try and identify which patients are being selected for

Table 17: BSBMT Indications for SCT in Mantle Cell Lymphoma (taken from BSBMT Indications for SCT Version Oct 13)

Mantle Cell Lymphoma

	Autograft	Sibling transplant	MUD transplant
CR1/PR1	S[1]	CO[2]	CO[2]
CR/PR>1	CO[3]	CO[3]	CO[3]
Chemorefractory (<PR)	GNR	GNR	GNR
Relapse post autograft	GNR	S[4]	S[4]

References

1. Dreyling et al Blood 2005; 105:2677-2684, Geisler et al Br J Haematol. 2012; 158: 355-62
2. Consider if high MIPI-B, high KI67+ fraction, patients with primary resistant disease requiring salvage to achieve first response: Maris et al Blood 2004; 104: 3535, Tam et al. Blood 2009; 113: 4144-4152, Le Gouill et al Ann Oncol 2012; 23: 2695-2703; current NCRN trial (Rule et al)
3. Consolidation with either modality is acceptable practice, though allogeneic transplantation should be considered standard if the predicted NRM is acceptable. Tam et al Blood 2009; 113: 4144-4152
4. Allogeneic transplantation should be considered standard in chemosensitive patients if the predicted NRM is acceptable. Robinson et al Blood 2002; 100: 4310-4316; 104: 2322, Faulkner et al Blood 2004; 103: 428-434, Tam et al. Blood 2009; 113: 4144-4152, Le Gouill et al Ann Oncol 2012; 23: 2695-2703

Table 18: BSBMT Indications for SCT in Peripheral T Cell Lymphoma (taken from BSBMT Indications for SCT Version Oct 13)

Peripheral T cell Lymphoma

	Autograft	Sibling transplant	MUD transplant
CR1/PR1	CO[1]	CO[2]	CO[2]
CR/PR>1	S[3]	CO[2]	CO[2]
Chemorefractory (<PR)	GNR	D	D
Primary resistant - Sensitive to salvage - Resistant	 S[4] GNR	 S[4] GNR	 S[4] GNR
Relapse post autograft	GNR	S[5]	S[5]

References

1. Consider for subtypes other than ALK$^+$ALCL with high or high-intermediate aaIPI score. Mounier et al JCO 2002; 20: 1790-1797, Rodriguez et al Ann Oncol 2003; 14: 1768-1775, Corradini et al Leukaemia 2006, 20: 1533-1538, Reimer et al JCO 2009: 27: 106-113, d'Amore et al Blood (ASH 2011); 118; 331
2. Consider appropriate timing according to histological subtype e.g. for hepatosplenic lymphomas there should be early consideration of allografting. Corradini et al JCO 2004; 22:2172-2176, Wulf et al BMT 2005; 36:271-273, Kyriakou et al JCO 2009; 27: 3951-3958, Shustov et al BJH 2010; 150: 170-178, Jacobsen et al Ann Oncol 2011; 22: 1608-1613, Dodero et al Leukemia 2012: 26: 520-526, Smith et al JCO 2013; 31: 3100-3109

allograft and whether any cohorts can be demonstrated to gain particular benefit. In view of the histological heterogeneity included in this cohort, my approach will focus on the three subtypes with the largest patient numbers; DLBCL (including transformed follicular lymphoma), FL and MCL. Following whole group analyses, histological subtypes will be considered and discussed individually.

5.2 Hypothesis

a) Reduced-intensity T replete allogeneic transplantation is a reasonable standard of care in those with resistant and relapsed follicular lymphoma and transformed FL/DLBCL

b) Reduced-intensity T replete allogeneic transplantation is a reasonable standard of care in MCL in CR1

5.3 Methods

5.3.1 Patient eligibility

A total of 72 patients with lymphoproliferative disorders underwent RIC-SCT between 2005 and 2013 at St Bartholomewõs Hospital. These included patients with DLBCL (including those transformed from FL), FL, PTCL, MCL, Waldenströms Macroglobulinaemia (WM), HL and marginal zone lymphoma (MZL). Written informed consent was obtained from all patients and donors in accordance with the Declaration of Helsinki.

5.3.2 Treatment protocol

Transplant conditioning was with fludarabine 25 mg/m^2 on Dī 6 to Dī 2 and cyclophosphamide 1 g/m^2 on Dī 3 and Dī 2. In five cases, the donor was an HLA single antigen mismatch and the recipient received Alemtuzumab in addition. Methotrexate (5 mg/m^2 on D+1, D+3 and D+6), together with ciclosporin (5 mg/kg on Dī 2 then 3 mg/kg per day from Dī 1, tailing at day +100 or sooner in the presence of mixed chimerism) was used for GVHD prophylaxis. Trough CSA levels were measured in whole blood twice weekly post-transplant. CSA was dosed to maintain levels between 150ï 300mcg/L.

5.3.3 Supportive care

All patients received antifungal and antimicrobial prophylaxis with fluconazole and ciprofloxacin until neutrophil engraftment. All patients were commenced on cotrimoxazole following engraftment as prophylaxis against *Pneumocystis jiroveci*. All patients received antiviral prophylaxis with aciclovir for a minimum of 6 months or longer if still on immunosuppression. Peripheral blood samples were monitored weekly for CMV reactivation by quantitative polymerase chain reaction (PCR). Patients with evidence of CMV re-activation were treated with pre-emptive valganciclovir.

5.3.4 Patient evaluation

Disease response was evaluated according to lymphoma consensus guidelines.[177, 178] PFS was measured from time of SCT date to date of progression. OS was calculated from SCT infusion date to date of death. NRM was defined as death in the absence of disease relapse or persistence. Acute GVHD was diagnosed and graded using current consensus criteria.[113] Chronic GvHD was diagnosed and scored using Seattle criteria.[114]

5.3.5 Statistical analysis

Survival curves were constructed using the method of Kaplan and Meier and differences between groups were assessed using the log-rank statistic. Patients were censored at last follow-up when calculating overall survival. Univariate analysis of association of outcomes with risk factors was performed. These included age at time of transplant (60 yrs or Ó60 yrs), HCT-CI, diagnosis, stage, number of prior treatment lines, previous autologous transplant, presence of chemosensitive disease, disease status at time of transplant, presence of refractoriness to Rituximab, donor type, donor age, sex mismatch, major ABO mismatch, CD34$^+$ and CD3$^+$ cell doses, CMV risk and the presence of acute and chronic GVHD. Where previous autologous transplantation had occurred, time to relapse and the interval between autologous and allogeneic transplant were evaluated.

All *P*-values were two-sided, and *P*-values of less than 0.05 were considered statistically significant. Data was analysed using SPSS v22 (IBM, New York).

5.4 Results

5.4.1 Patient characteristics

Patient, donor and graft characteristics are summarized in table 19. The median age of the patient cohort was 53 years (range 22-66 years). Nineteen patients (26.4%) were aged 60 years or over. Twenty patients (27.8%) had FL. 20.1%, 16.7% and 15% of patients had MCL, DLBCL (including transformed FL) and WM respectively. The remainder included HL, PTCL and MZL. Further detail on patient characteristics in those with DLBCL, FL and MCL are shown in tables 20, 21 and 22 respectively. Median follow-up for surviving patients was 58.5 months.

Patients were transplanted using HLA-matched related donors (n=33, 45.8%), matched VUDs (n=34, 47.2%) or mismatched VUDs (n=5, 6.9%). There was a major ABO mismatch in 19 cases (27.9%). In 61.8% of cases, there was a CMV reactivation risk. There were 17 cases of a male recipient and female donor (23.6%). Median donor age was 42 years (range 22-68 years).

Intermediate resolution HLA-typing was used for Class I (HLA-A and HLA-B, HLA-C) and high resolution molecular typing for Class II (DRB1 and DQB1) alleles. Stem cell source was granulocyte colony-stimulating factor-mobilized PBSCs in all cases. Patients received a median CD34$^+$ cell dose of 5.68 x 10^6/kg (range 0.81-44.88) and median CD3$^+$ T cell dose of 2.66 x 10^8/kg (range 0.27-8.82).

Table 19: Patient characteristics undergoing allogeneic transplant for lymphoproliferative disorders

		N	%
Diagnosis	**DLBCL**	12	16.7
	FL	20	27.8
	WM	11	15.3
	MCL	15	20.8
	PTCL	5	6.9
	HD	6	8.3
	MZL	3	4.2
Gender	**M**	42	58.3
	F	30	41.7
Age	**Med (range)**		53 (22-66)
Stage	**1**	1	1.4
	2	2	2.8
	3	13	18
	4	49	68.1
	NA	7	9.7
HCI-CI	**Low**	32	44.4
	Int	25	34.7
	High	15	20.8
Lines of treatment	**Med (range)**		4 (1-8)
Previous ASCT	**Y**	29	40.3
	N	43	59.7
Treatment lines post ASCT	**Med (range)**		1 (0-4)
Relapse time post ASCT	**<12 m**	2	9
	13-24 m	4	18.2
	>24 m	16	72.7
Chemosensitive		48	66.7
Chemorefractory		14	19.4
	NA	10	13.9
Response pre RIC-SCT	**CR**	38	52.8
	<CR	34	47.2
Rituximab refractory		15(of 50 who received Rituximab)	30
Year of diagnosis (excluding PTCL & HD)	**Pre 2003**	26	59
	Post 2003	25	41
Donor	**Matched SIB**	33	45.8
	Matched VUD	34	47.2
	Mismatched VUD	5	6.9
Major Blood Gp Mismatch	**Y**	19	26.4
	N	49	68.1
	NA	3	4.2
Female donor/male recipient		17	23.6
Donor age median (range)	**MUD**		32.5 (20-44)
	SIB		53 (27-77)

NA not available

Table 20: Patient characteristics of those with DLBCL(n=12)

		%
Age≥ 60 years	Y	25
	N	75
HCT-CI	Low	33.3
	Int	58.3
	High	8.3
Previous Autograft	Y	66.6
	N	33.3
Response pre allograft	CR	50
	<CR	50
Rituximab refractory	Y	25
	N	75
Chemosensitive	Y	75
	N	25
Lines of treatment (median)[range]		5[3-8]
Duration of last treatment response (months)	<12 mths	8.3
	13-24	33.3
	>24	50
	No durable response	16.7

Table 21: Patient characteristics of those with follicular lymphoma(n=20)

		%
Age≥ 60 years	Y	20
	N	80
HCT-CI	Low	45
	Int	30
	High	25
Previous Autograft	Y	45
	N	55
Response pre allograft	CR	55
	<CR	45
Rituximab refractory	Y	45
	N	55
Chemosensitive	Y	75
	N	25
Lines of treatment (median)[range]		4[3-8]
Duration of last treatment response (months)	<12 mths	25
	13-24	5
	>24	55
	No durable response	15

Table 22: Patient characteristics of those with mantle cell lymphoma(n=15)

		%
Age≥ 60 years	Y	40
	N	60
HCT-CI	Low	66.7
	Int	20
	High	13.3
Previous Autograft	Y	26.7
	N	73.3
Response pre allograft	CR1	33.3
	PR1/VGPR1	13.3
	CR2	46.7
	<CR2	6.7
Rituximab refractory	Y	16.7
	N	83.3
Chemosensitive	Y	86.7
	N	13.3
Lines of treatment (median)[range]		2[1-6]
Duration of last treatment response (months)	<12 mths	11.1
	13-24	11.1
	>24	66.7
	No durable response	11.1

5.4.2 Engraftment and chimerism

Excluding those who died before engraftment, all other patients engrafted and median time to neutrophil engraftment was 15 days (range 11-29). Median time to platelet engraftment ($>20x10^9$) was 12 days. FDC was achieved in 84.3% of all assessable patients. Median time to achieve FDC of whole blood was 90 days.

5.4.3 Toxicity and non-relapse mortality

NRM at 100 days was 12.5% with a 3-year NRM of 25%. 47.6% of all deaths were secondary to GVHD, the majority of which were associated with infection related to immunosuppression. 38% of deaths were due to infection and 3 cases (14.3%) were due to other causes including intracerebral haemorrhage and renal failure. 79% of treatment-related deaths were in patients above the age of 50, with over half of those being in those over 60 years (42.8% of all NRM deaths). Consistent with the overall study population, forty percent of deaths in those over 60 years were secondary to GVHD and 40% were secondary to infective causes. However, 50% of deaths in those aged over 60 occurred within the first 100 days post transplant. Thirteen patients had CMV reactivation (18%) but there were no cases of CMV related disease. No other significant viral infections occurred.

5.4.4 Relapse & use of DLI

Twelve patients (16.7%) relapsed post transplant. Median time to relapse was 25.5 months. Of these, 7 patients were treated with DLI which resulted in a CR being achieved again in all cases. Six of these patients are still alive and one patient died of sepsis. DLI was utilised in patients with diagnoses of FL (n=1), DLBCL (n=2), PTCL (n=1), MCL (n=1) and WM (n=2), demonstrating evidence of the graft-vs-lymphoma effect in all of these histological subtypes. Univariate analysis did not identify any pre or post transplant factors that impacted on relapse risk. The small number of relapse events may have contributed to the failure to identify any significant differences.

5.4.5 Survival

Five year PFS was 52%. Five-year OS was 60% for all patients. There was a clear plateau, with the overall survival remaining at 60% 10 years post transplant. Whilst survival differences were observed between different histological subtypes (5 year OS at 40% for PTCL and 78.6% for DLBCL), these were not statistically significant for either PFS or OS, with analysis limited by small patient numbers in some diagnostic categories; (figures 23 & 24).

Figure 23: Overall survival in lymphoma allogeneic transplants by diagnostic groups. Indolent lymphoma includes FL, WM and MZL

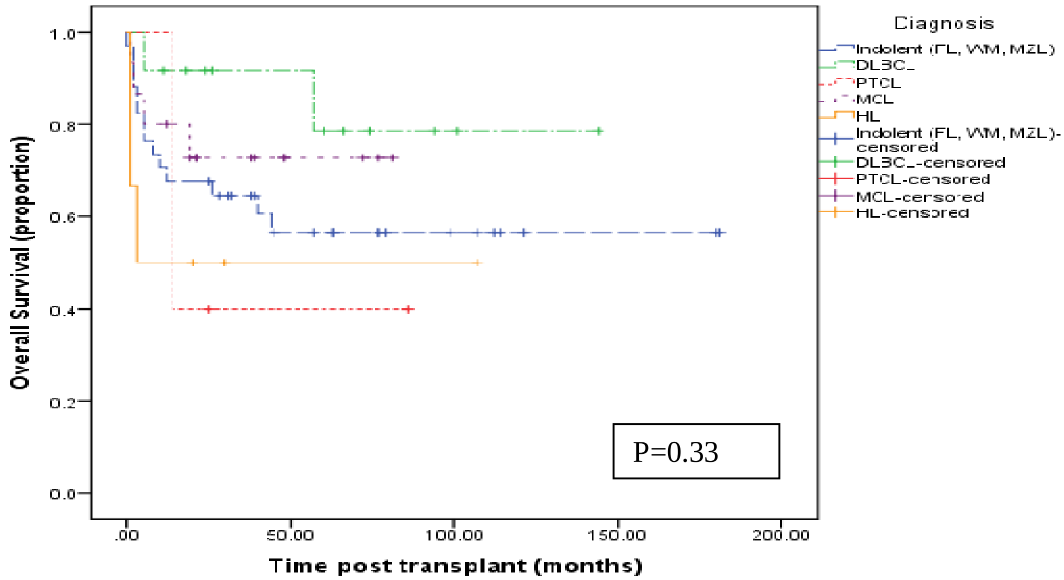
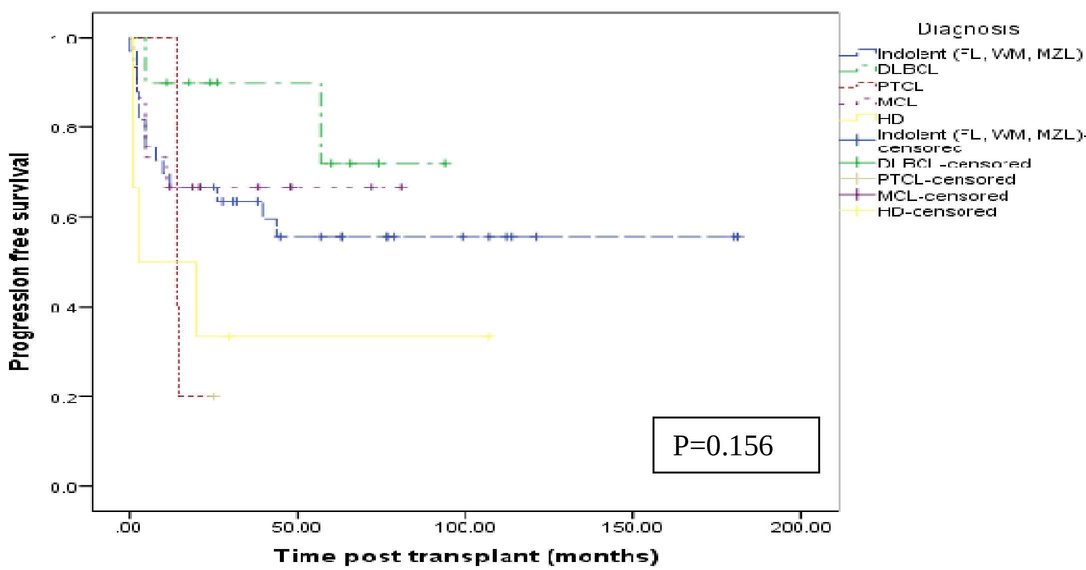


Figure 24: Progression free survival in lymphoma allogeneic transplants by diagnostic groups. Indolent lymphoma includes FL, WM and MZL



With regards to pre-transplant variables, age had a significant impact on OS with 5 year OS of those above and below 60 years at 50% and 70% respectively; p=0.015 (figure 25). Whilst the PFS Kaplan-Meier demonstrated an almost identical trend, age did not quite reach statistical significance; p=0.08 (figure 26). Although numbers were smaller, those aged under 40 (n=7) had a 5 year OS over 80%, an interesting observation but too small to draw conclusions from.

Figure 25: Overall survival in lymphoma allogeneic transplants by age above and below 60 years

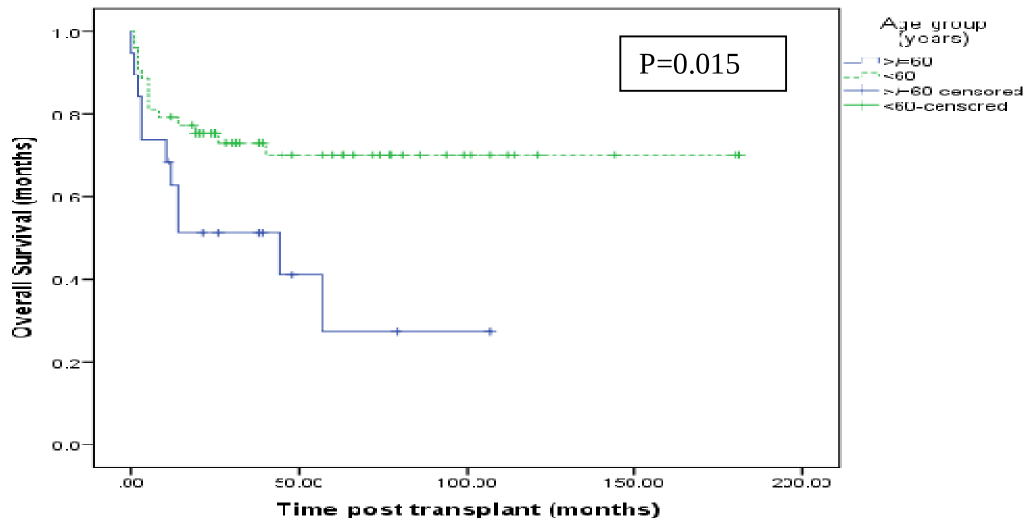
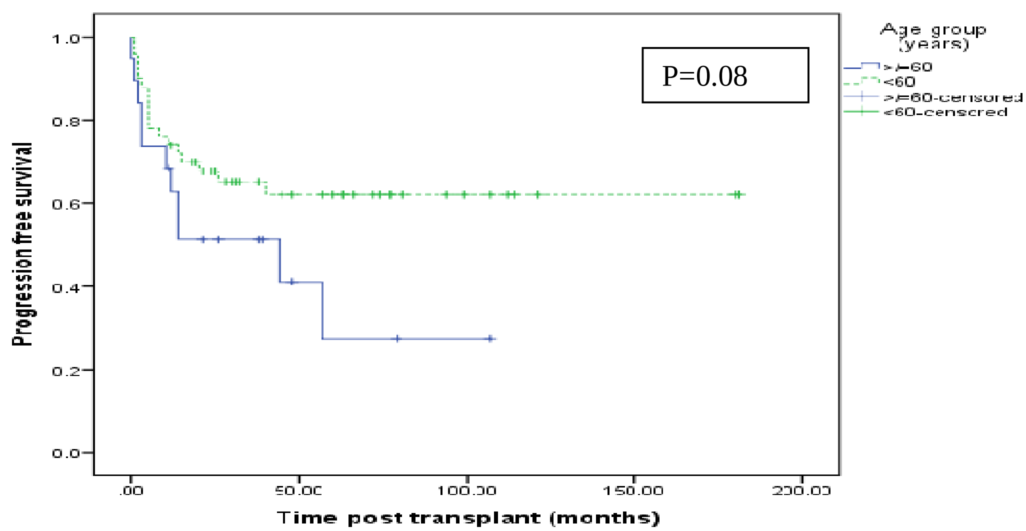


Figure 26: Progression free survival in lymphoma allogeneic transplants by age above and below 60 years



Disease response prior to transplant was significant with 5 yr OS at 67% vs 47% for those in a CR at the time of transplant compared to any response less than this respectively; p=0.044 (figure 27). Almost identical findings were seen with PFS; p=0.045 (figure 28).

Figure 27: Overall survival in lymphoma allogeneic transplants according to pre-transplant response

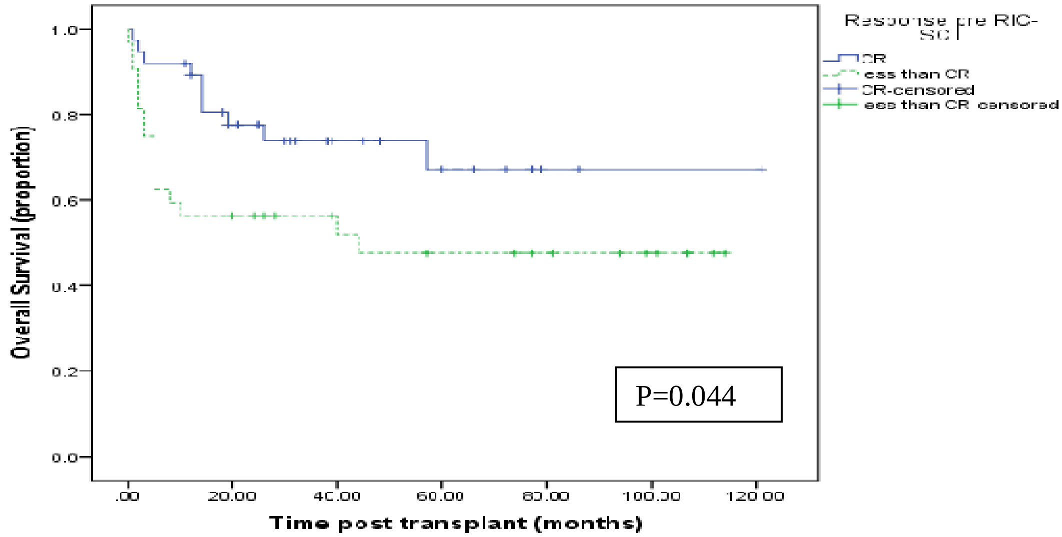
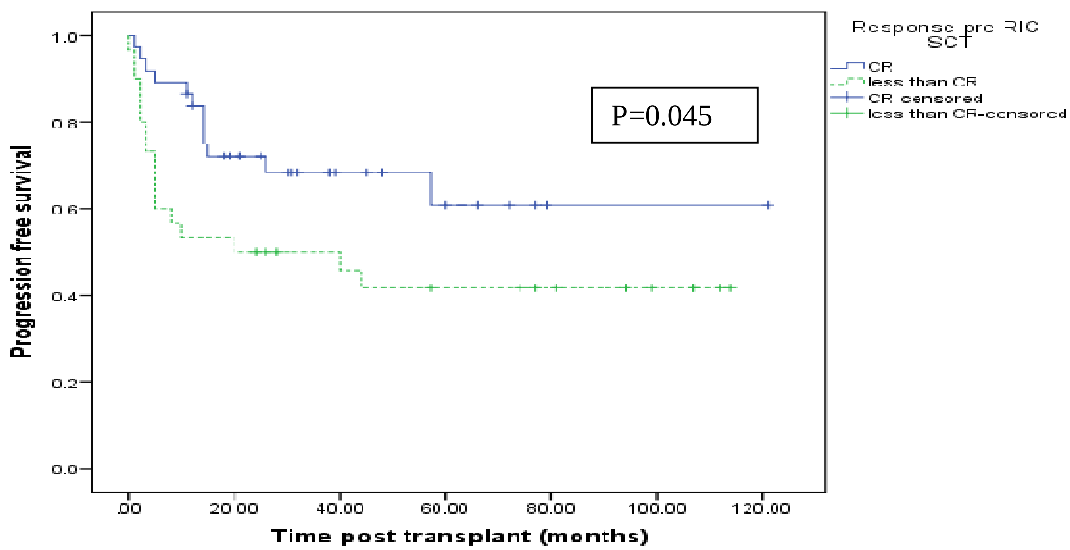


Figure 28: Progression free survival in lymphoma allogeneic transplants according to pre-transplant response



In conjunction with this, patients who had chemosensitive disease at the time of transplant had significantly better OS than those with chemoresistant disease (5 year OS 65% vs 40%; p=0.049 (figure 29). The trend was replicated in PFS, although significance was not reached; p=0.061 (figure 30).

Figure 29: Overall survival in lymphoma allogeneic transplants according to chemosensitive vs chemoresistant disease at the time of transplant. Chemoresistant disease is defined as achieving less than a partial response with the last line of treatment prior to transplant.

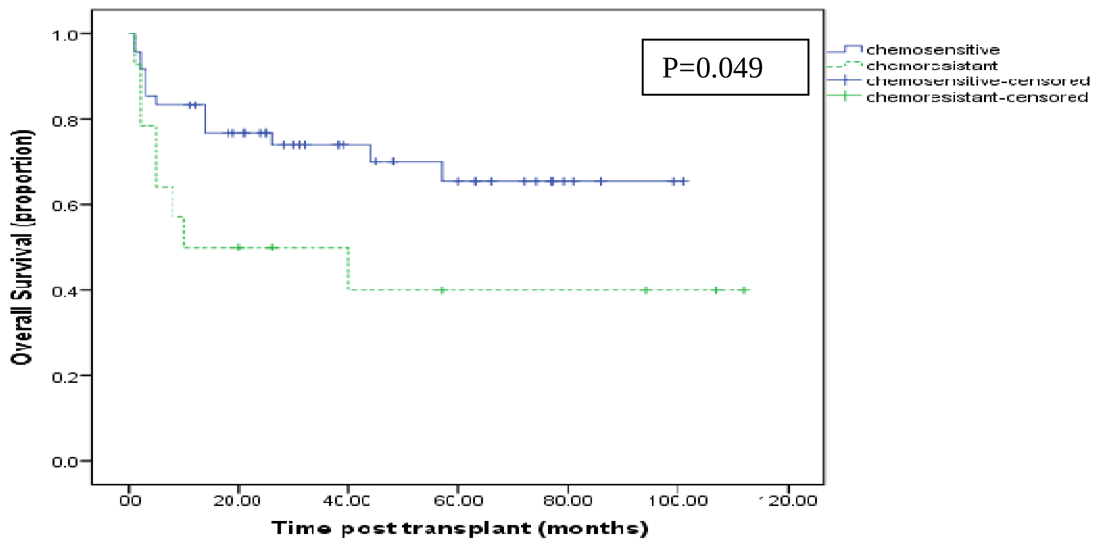
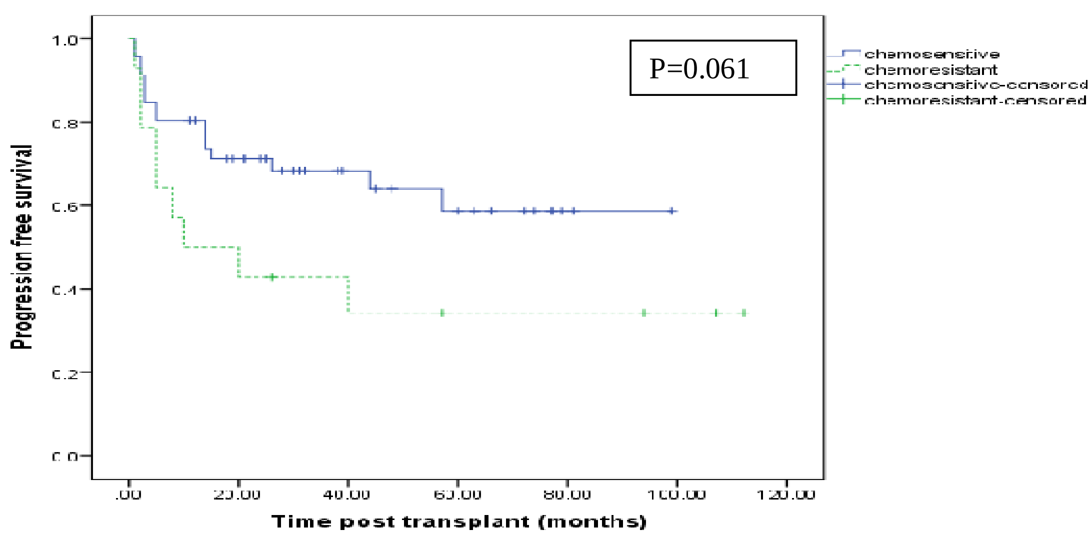


Figure 30: Progression free survival in lymphoma allogeneic transplants according to chemosensitive vs chemoresistant disease at the time of transplant.

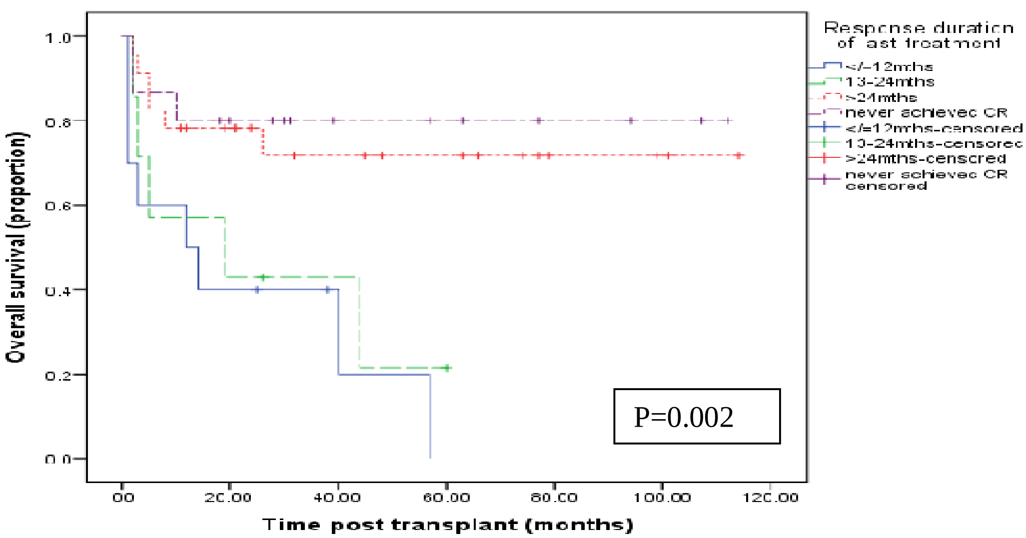


Having primary refractory disease or previous refractoriness to Rituximab (in those who had received it) had no impact on OS. Patients in whom treatment with Rituximab upfront would be considered standard of care in today's practice were divided into two groups based upon the date of their diagnoses being before or after the introduction of Rituximab into standard practice. Five-year OS was nearly 80% in the Rituximab group compared to less than 60%

in those who did not receive it upfront. However, there was no statistical significance in OS between the two groups.

In those patients who were undergoing an allogeneic transplant having relapsed post autologous transplant, time to relapse post autologous transplant had a significant impact on OS. Neither patient who relapsed within 12 months of autologous transplant survived (n=2) compared to a 5 year OS of 50% and 64% for those with relapse between 12-24months and greater than 2 years respectively (p<0.0001). Similarly, and perhaps more strikingly, if all patients were considered in terms of their last response time prior to salvage treatment and allograft, 5 year OS was 0%, 21.4% and 71.7% for those with a response time less than 12 months, 12-24 months and greater that 24 months respectively; p=0.002 (figure 31). The same observation was seen with PFS (p=0.006).

Figure 31: Overall survival in lymphoma allogeneic transplants by duration of last treatment response prior to transplant. Patients treated in CR1 excluded.



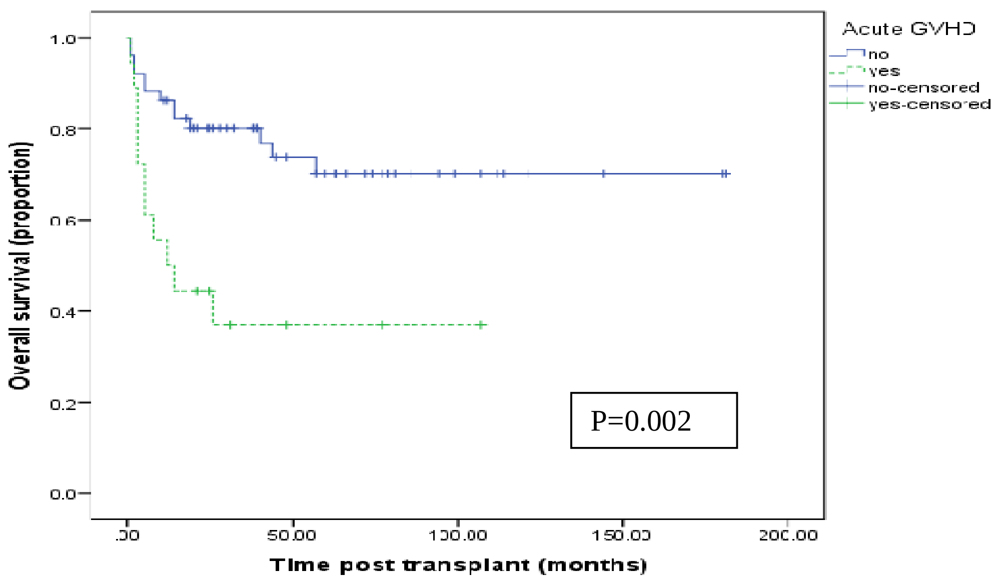
The cohort of patients who had never achieved a sustained CR following any line of therapy prior to allogeneic transplant had a surprisingly good outcome with a 5 year OS of 80%.

There was no impact of gender, HCT-CI, previous autologous transplant, number of lines of treatment in total or post autologous transplant, donor type, donor age, blood group mismatch, gender mismatch or CMV risk or reactivation on relapse, PFS or OS. CD34 and CD3 count had no significant effect on relapse, PFS or OS. Achievement of FDC significantly improved OS; p<0.0001.

5.4.6 Graft vs Host Disease

Eighteen (26.1%) of all evaluable patients experienced acute GVHD (grades II-IV) of whom 28% (n=5) died from GVHD within 100 days. Median time to acute GVHD was 31.5 days (range 19-76 days). Of those who developed acute GVHD, 11 (61%) died in total; 8 within the first year from transplant. Cause of death was GVHD or infection in the context of systemic immunosuppression in all cases. A significantly worse outcome for those who developed acute GVHD (grades II-IV) was seen, with a 5 year OS of 37% vs 70% for those with and without acute GVHD; p=0.002 (figure 32). The same observation was seen for PFS; p=0.002.

Figure 32: Overall survival in lymphoma allogeneic transplant by occurrence of acute GVHD



43 patients (69%) of all assessable patients had chronic GVHD or GVHD on tailing ciclosporin and 13.9% of these died from GVHD or infection associated with GVHD treatment. Occurrence of chronic GVHD demonstrated a non-significant trend for improved OS (5 year OS of 73% vs 62%; p=0.168).

5.5 Discussion

The utilisation of RIC-SCT in lymphoma offers potential long term remission and its use is growing. However its role in terms of which patients are most suitable, prognostic factors to aid patient selection and when it should be used remains a subject of debate. This retrospective analysis of 72 patients with a mix of histological subtypes, using T replete conditioning, reports a 5 year OS and PFS of 60% and 52% respectively. 100 day NRM was 12.5% which is consistent with the BSBMT data of 12% in the NHL cohort. Three year NRM was 25%. These results are consistent with other reports that have evaluated a heterogeneous lymphoma population and again confirm RIC-SCT as a viable and potentially curative treatment strategy in a heavily pre-treated group who otherwise carry a relatively poor prognosis.[179, 180]

Acute and chronic GVHD that required treatment with immunosuppression occurred in 26% and 69% of all patients. Despite this relatively high incidence, overall NRM was not higher than other studies that utilised T deplete conditioning.[179] Whilst DLI was used with success in 7 patients, this conditioning platform places much less reliance on DLI as a therapeutic strategy, which is important where over 50% of donors were unrelated.

Several prognostic pre-transplant factors were identified to be predictive of survival outcomes in this analysis. These included age above 60 years, disease status at transplant, chemosensitivity at time of transplant and duration of last response prior to allograft. These factors should certainly be taken into account when considering patient selection. Whilst having chemoresistant disease and being in less than a CR at the time of transplant have inferior survival outcomes, there is still a survival plateau for both these groups. Therefore a proportion of patients will still be cured and a discussion between clinician and patient should occur in such scenarios to consider the relative merits of a RIC-SCT over other options. In those patients above 60 years with short response times prior to transplant there is no plateau evident in the survival curves making the potential benefit of RIC-SCT more questionable.

The biggest limitation of this retrospective analysis is the heterogeneous population, both of lymphoma subtypes and indeed patient characteristics. It is well recognised that different diseases respond differently to RIC-SCT and that patients with better disease characteristics are expected to have superior outcomes. To compare them *en masse* provides evidence of the utility of this conditioning platform but provides little valid information for directing ongoing decision making within each subtype. Some groups have particularly small numbers making analysis for them alone statistically non-viable. However, I will now discuss the three largest subtypes individually; namely DLBCL, FL and MCL.

5.5.1 Diffuse large B-cell lymphoma

DLBCL is the commonest histological subtype of NHL. First line treatment with R-CHOP chemotherapy is considered the standard of care for those considered fit to receive intensive treatment resulting in a reported overall survival of 70-93% and an event-free survival of 61% to 79%.[181-183] For those patients that relapse after successful initial treatment or those that do not achieve a CR with first-line therapy, salvage chemotherapy followed by an autologous stem cell transplant is the standard of care.[165] This is supported by the BSBMT recommendations (table 15). Using this approach, progression free survival for patients who have previously been treated with or without Rituximab is approximately 50% at 3 years. However, the outcomes are significantly worse if relapse has occurred within 12 months of treatment with Rituximab with a 3 year PFS of 23% in this group.[184]

The role of allogeneic transplantation in DLBCL is less clear. The efficacy, at least in part, of allogeneic transplantation is attributed to the immune mediated graft-versus-lymphoma effect. There has been some doubt cast on how significant this is in DLBCL following reports that there is no significant reduction in relapse compared to autologous transplantation.[166, 185] However, the interpretation of studies where equal relapse rates were reported is muddied by unevenly matched patient groups with a worse cohort of patients receiving an allogeneic transplant, perhaps offsetting the potential benefits of GvL. Other studies have provided support for the demonstration of GvL in this sub-type of lymphoma with a reduction in relapse rates compared to those patients undergoing autologous transplantation.[186, 187] The successful use of immunosuppresion withdrawal and DLI to achieve disease control is further evidence of GvL in DLBCL.[170, 171, 179] With unacceptably high TRM rates with myeloablative conditioning,[166, 185] the focus is now on RIC-SCT and where this is placed in the treatment of DLBCL.

This study included 12 patients with DLBCL. Table 20 summarises their characteristics. Ten of these were patients that had transformed from follicular lymphoma. 75% had undergone a previous autologous transplant. 25% were over the age of 60 years. The median number of treatment lines prior to transplant was 5. 75% had chemosensitive disease at the time of transplant and 50% were in CR. Five year OS was 78.6% and three patients (25%) relapsed or progressed following transplant. Of these, 1 died of disease, the other 2 both achieved CR following DLI administration and are long term survivors. There were 2 post transplant deaths, one secondary to progressive disease as already mentioned; the other was a late death at 57 months secondary to the development of nephrotic syndrome and renal failure, the aetiology of which was unclear.

This data is favourable compared to the literature where TRM is reported at between 20%-39% at between 2 and 5 years. Overall survival for RIC-SCT in patients with DLBCL is 26-52% at 3 to 5 years with a PFS of 25-48%.[171, 188-192] It is unclear why there is such a discrepancy between our data and the literature; certainly in terms of patient characteristics the cohort seems comparable. It is a small cohort and perhaps if this group was expanded, the outcome data would change. Is it the impact of being a group made up of predominantly transformed FL, rather than *de novo* DLBCL? This cohort has not been identified to have superior outcomes in the literature but given that there is stronger evidence for a graft-vs-lymphoma effect in FL, perhaps this in turn is responsible for the improved responses seen in this cohort in the RIC-SCT setting. There has been speculation that the GVL effect in DLBCL may be limited but the superior OS in this study is supported by the occurrence of cGVHD in 10 patients (83%) and a better OS in those with GVHD. The successful use of DLI in 2 patients is further evidence of the GVL effect in DLBCL.[188, 193-195]

Some studies have demonstrated that patients with indolent NHL do better that those with aggressive subtypes after RIC-SCT.[179, 196] However, this study demonstrated that the DLBCL cohort had a superior, although not significant, OS compared to the indolent lymphomas (FL,WM and MZL) with a 5 year OS of 78.6% vs 56.6% respectively (p=0.138), an outcome that again may be confounded by the make-up of the DLBCL cohort.

So where is allogeneic transplantation best placed in this lymphoma subtype? BSBMT guidelines recommend it as standard of care in those who are primary resistant but sensitive to salvage and in those who have relapsed post autograft. It is a clinical option to consolidate responses at relapse with allograft rather than autograft (table 15).

Following the introduction of Rituximab to upfront DLBCL treatment, response rates with chemotherapy are improving and fewer relapses are occurring. However, when relapses do occur, especially if they occur early, autologous transplantation is less likely to offer benefit.[184] Perhaps consideration should be given to this cohort of patients being taken straight to an allograft if a donor is available. There is however no evidence to support this strategy. In the cohort analysed in this study, four patients underwent RIC-SCT without having undergone a previous ASCT. One of these failed to harvest stem cells, another had poor cardiac function felt to be inadequate to safely proceed with an ASCT. The remaining two were both patients with previous FL who had never achieved a CR despite multiple lines of therapy and therefore never felt to have good enough disease control to proceed with an ASCT. This is clearly too small a group to analyse with regards to outcomes, although these cases highlight scenarios where RIC-SCT may be especially useful.

An algorithm for who should be considered for allograft vs autograft has been proposed using a similar rationale whereby not only those who have relapsed post autograft but also those who are not likely to benefit from an autograft should be considered as allograft candidates.[197] However, clearly more data is needed to support this as a valid treatment pathway given the significant associated NRM.

5.5.2 Follicular lymphoma

Follicular lymphoma is an indolent lymphoma with many patients surviving for decades following diagnosis. Despite the introduction of Rituximab to first line treatments, which have undoubtedly improved outcomes,[198, 199] there remains a cohort of patients with a more aggressive form of the disease who are more resistant to conventional chemotherapy and carry a worse prognosis.[200, 201]

For those patients who relapse, salvage chemotherapy followed by an autologous stem cell transplant is considered the standard of care.[164] This is supported by the BSBMT guidelines and studies have demonstrated an improved PFS and OS compared to chemotherapy alone (table 14). Studies in the pre-Rituximab era reported OS between 50-70% depending on the time point and study.[202-204] More relevant to today's practice, in those who have received Rituximab upfront and as part of salvage treatment, 3 year OS post ASCT has been reported as 92% compared to 63% in those who received salvage chemotherapy alone at relapse.[205] However, whilst a small cohort of patients have extremely long progression-free survival post autograft and may well be cured, as a general principle this transplantation strategy is not considered a curative one for the majority, and relapse or progression is the main cause of treatment failure.[204]

The graft versus lymphoma effect in FL has been well demonstrated.[172, 194, 195] As in other haematological malignancies, the reduction in disease relapse seen in early studies utilising myeloablative conditioning was offset by high TRM rates resulting in no OS benefit.[167, 206] However the role of RIC-SCT in FL is growing. It is the only definitive curative strategy and studies report PFS ranging between 43% and 76% and OS from 52% to 81%. TRM has been reported as ranging between 11% and 42% at 3 years.[179, 188, 207-209] These wide-ranging outcomes most likely stem from the differences in the study designs with all utilising different conditioning regimens, including differing patient cohorts and follow-up times.

Most studies that have evaluated the outcomes of FL patients with RIC-SCT have included a proportion of patients who have had a previous autograft for whom the outcomes are worse.[207, 209] However, the BSBMT guidelines recommend a RIC-SCT as a standard of care in those patients who have relapsed following autograft, if the patient is considered a suitable candidate. The role of allograft rather than an autograft in consolidating treatment of salvage chemotherapy-responsive relapsed disease is more controversial.

A prospective study which attempted to directly compare the outcomes of autograft vs RIC-SCT at first relapse closed early due to poor recruitment.[210] A retrospective study comparing the two treatment strategies concluded that whilst RIC-SCT was associated with a higher NRM and reduced relapse rate, there was no overall difference in OS.[211] However, the two patient cohorts were clearly subject to selection bias and were not equally matched either at the point of transplant or in their subsequent management. Therefore it is very difficult to draw any valid conclusions about how the two strategies compare.

BSBMT recommends RIC-SCT in first relapse, with either a sibling or MUD, as a ©clinical optionô It states it may be especially appropriate in those who are considered at high risk of failure from an autograft which includes those who have had a short first response, a high FLIPI at relapse or who have failed to achieve a CR with salvage chemotherapy (table 14). So whilst it may be true that those who have not achieved a CR will not do so well with an autograft and may derive most benefit from an allograft, it is likely that the patients that are chemosensitive are the ones who will benefit most from either transplant strategy.

So where does this leave us with regards to our own practice and patient selection? Practice at St Bartholomewos Hospital is in line with BSBMT recommendations. Patients undergo an ASCT at relapse and if it is felt appropriate and a suitable donor available, are considered for a RIC-SCT if relapse occurs post ASCT. Table 21 summarises the characteristics of the 20 patients with FL who underwent RIC-SCT. 20% were over the age of 60 years. 55% were in CR at the time of RIC-SCT, 45% were Rituximab refractory and 75% had chemosensitive disease at the time of transplant. They had undergone a median of 4 lines of treatment prior to allogeneic transplant. 45% had undergone a previous ASCT, a surprisingly low proportion, given that the standard of care is to perform an allogeneic transplant following relapse after ASCT. Looking at this group in more detail, 1 patient failed to harvest, 1 patient had an allogeneic transplant for FCR-induced aplasia and 1 patient elected not to have an ASCT previously, The remainder were all patients who had undergone multiple lines of treatment, most of whom had never achieved a CR and two who had very short responses following Rituximab-based chemotherapy, who were perhaps felt unlikely to sustain long-term benefit from an ASCT.

The 5 year OS for FL patients was 57.4% with only 1 patient relapsing. 3 year NRM was 30%, although small patient numbers may have adversely impacted upon this. Numbers were small to adequately assess the impact of pre-transplant variables. However, donor type did significantly impact upon outcome with 5year OS for sibling vs MUD 87.5% vs 32% respectively (p=0.042).

Data submitted to BSBMT based upon autologous transplants performed in NHL at St Bartholomewos shows a 5 year OS of 57% for all NHLs. These outcomes are similar to those demonstrated with the RIC-SCT approach. The autologous transplant patient cohort is mixed, including other NHLs and clearly these 2 datasets cannot be directly compared. However, if the outcomes really are so similar at the 5 year mark, then perhaps the prospect of long-term cure, at least for a proportion, makes the role of RIC-SCT seem more attractive.

Returning to consider the first hypothesis of this chapter, it is certainly reasonable to place RIC-SCT as standard of care if relapse occurs post ASCT and therefore support the hypothesis. Analysis of this data has highlighted the question of which patients should be consolidated at first relapse with a RIC-SCT over an ASCT. Practical issues such as failed stem cell harvests and suboptimal organ function for ASCT make RIC-SCT a reasonable choice in this scenario. However, in terms of disease stratification, whilst suggestions have been made, to date there remains inadequate evidence in the literature to suggest that this should be standard of care, and for now, it therefore should remain as a clinical option.

5.5.3 Mantle cell lymphoma

Mantle cell lymphoma is a much less common subtype of NHL, accounting for 6% of all lymphomas. Over the last decade, the introduction of Rituximab, high dose chemotherapy regimens and the use of consolidation with autograft in first response, have improved outcomes with median OS reported as over 7 years.[212, 213] However, these strategies are not curative and relapses continue to occur.

As in other lymphoproliferative disorders, the role of allogeneic transplantation is growing but its place remains controversial. There has been clear demonstration of the GvL effect in MCL with successful use of immunosuppression withdrawal and DLI to eradicate disease post transplant and the simultaneous occurrence of GVHD and disease response.[214, 215] Again, as with other lymphoma subtypes, the use of myeloablative allogeneic transplantation has not demonstrated a survival benefit, most likely because of the high associated NRM.[168] Consequently, the focus again is on the role of RIC-SCT.

Transplant strategy at St Bartholomewos is to consolidate patients with MCL with an ASCT in first response. However, in those who are young, fit and who demonstrate poor disease characteristics (e.g. blastoid variant or high ki67) then a donor search would be commenced during first line therapy and the patient would be considered for a RIC-SCT in CR1. The characteristics of the 15 patients with MCL who underwent RIC-SCT are shown in table 22. Using this T-replete conditioning regimen, 5 year OS based upon 15 patients was 72.7%.

This compares favourably to other low grade lymphomas within this analysis and also to the literature which reports OS as between 37% to 62%.[179, 215-221] PFS was 67% at 5 years. All relapses (n=3) occurred within 12 months post transplant and no late relapses have been observed. 1 patient received DLI and achieved disease control; the other 2 patients died from progressive disease. Cumulative NRM in this subset of MCL patients was 13% at 3 years. Whilst low early NRM has been reported in some studies[215, 217] the majority of studies report a 2-3 year cumulative NRM between 20-32%.[179, 216, 218-220] Interestingly 7 patients underwent RIC-SCT in first response. Of the remainder (n=8) who were transplanted at relapse, 4 underwent an ASCT at first relapse, 2 declined an ASCT at first relapse and 2 proceeded with a RIC-SCT as their consolidation strategy following salvage therapy.

This analysis suggests that RIC-SCT with this conditioning regimen is a viable treatment strategy with favourable outcomes compared to both other NHL subtypes within our own centre and to other studies. However, the small numbers must be remembered, especially when comparing to studies with much larger patient cohorts.

Are these results secondary to patient selection? This retrospective analysis clearly has a selection bias in the patients being put forward for a treatment strategy not considered standard of care and known to carry a high NRM. The median age of patients in this group was 58 years, comparable to other studies. However, the cohort had 6 patients over the age of 60, but 66% with a HCT-CI of 0. This demonstrates that clearly patient selection is important in achieving good outcomes and that this is not by selection of age alone. Four patients (27%) had undergone a previous autograft and this had no impact on OS which is consistent with most studies. Median number of treatment lines prior to allograft was 2 (range 1-6), which is certainly lower than other reported studies and may be a factor in these favourable results. Those patients who had fewer than 3 treatment lines had 100% OS at 5 years compared to 33% for those with Ó3 lines (p=0.045), consistent with similar observations in other, although not all, studies.[216, 217] Twelve patients (80%) were in CR at the time of transplant. There was a trend to improved OS for this cohort compared with lesser responses with 5 year OS at 82.5% vs 33% (p=0.068). Of the 3 patients not in CR, 2 had early death secondary to progressive disease. Similarly, chemosensitivity predicted for superior outcomes with a 5 year OS of 91% vs 0% (p=0.005). Twelve patients had prior exposure to Rituximab. Two were Rituximab refractory and were deemed chemoresistant at the time of transplant. Both died of progressive disease within 6 months of transplant. Just under half had a matched unrelated donor. This is not identified as a prognostic factor in most studies.[222] Although there was a difference between survival outcomes between donor types (88% vs 57% 5 year OS for sibling and VUD respectively) this was not significant (p=0.133).

Whilst RIC-SCT may well be a reasonable treatment option, the literature remains unclear regarding which patients should be selected for allograft over autograft. The largest study performed by the Centre for International Blood and Marrow Transplant Research (CIBTMR) is a retrospective, non-randomised analysis of patients with chemosensitive disease undergoing ASCT or RIC-SCT either early (having received 2 or less lines of treatment) or later on in their disease. No differences in overall survival between ASCT or RIC-SCT were seen, mainly due to the higher TRM of the RIC allografts (25% vs 3%). Treatment with either transplant modality produced better outcomes when performed earlier in the disease course. They concluded that in the absence of a survival benefit, patients in first response should have an ASCT. Those who are considered to carry a lower TRM risk (by virtue of a lower HCT-CI) or who are at greater risk of disease relapse, should be considered for a RIC-SCT in first response.[219] This is consistent with the BSBMT and EBMT guidelines which list allogeneic transplantation as a óclinical optionô in patients with a CR/PR1 or greater, suggesting it is most appropriate in those with high risk disease or with an óacceptableô predicted NRM (table 17).[211]

In a disease that retains a high likelihood of relapse following autologous transplantation, the key to prolonged survival is RIC-SCT. If patient selection can be optimised to keep NRM down, it has the potential to further improve survival for at least a cohort of patients with this disease. This analysis would suggest that patients with chemosensitive disease, in CR, who are not heavily pre-treated are the most likely to benefit.

To return to the second hypothesis in this chapter, the numbers available in this retrospective analysis do not provide sufficiently strong data to support RIC-SCT being standard of care in CR1, and therefore the hypothesis must be rejected, for now. However, the outcome data in this lymphoma subtype is very good and this success appears to be secondary to a combination of good patient selection and transplanting early in the disease. If the same outcomes were replicated with larger numbers, this would provide a strong rationale for RIC-SCT to become standard of care in CR1 for MCL.

5.5.4 Waldenströms Macroglobulinaemia

WM is a rare lymphoproliferative disorder with an indolent course for the majority. A retrospective analysis identified that only 21% of patients required treatment at 100 months following diagnosis.[223] When treatment is required, response rates to combination chemotherapy are high and even at relapse, good responses may be achieved again. However, ultimately the disease will become resistant to conventional chemotherapy. Furthermore, prognostic stratification tools have identified the heterogeneity of this disease with a less indolent subgroup that carries a 5 yr OS of 36%.[224]

Its rarity, in combination with identifying patients who are suitable for transplantation means that evidence is limited with regards to the role of stem cell transplant. Unlike the lymphoma subtypes discussed previously, autologous transplantation is not recognised as a standard treatment strategy in those with relapsed or resistant disease. Rather, both autologous and allogeneic transplantation are considered a óclinical optionô in these scenarios (table 23).[169, 225]

Table 23: BSBMT Indications for Waldenströms Macroglobulinaemia (taken from BSBMT indications for SCT version Oct 13)

Lymphoplasmacytic Lymphoma/Waldenström's macroglobulinaemia

	Autograft	Sibling transplant	MUD transplant
CR/PR1	D[1]	GNR	GNR
CR/PR>1	CO[2]	CO[3]	CO[3]
Chemorefractory (<PR)	GNR	GNR	GNR
Primary resistant - **Sensitive to salvage** - **Resistant**	 CO[2] GNR	 CO[3] GNR	 CO[3] GNR

References
1. Dreger P et al Biol Blood Marrow Transplant 2007; 13:623-624, Caravita et al BMT 2009; 43:587-588
2. Kyriakou et al JCO 2010; 28:2227-2232, Tournilhac et al Semin Oncol. 2003; 30:291-296, Dhedin et al Haematologica 2007; 92:228
3. Garnier et al Haematologica 2010; 95:950-955, Kyriakou C et al JCO 2010; 28:4926-4934, Gilleece *et al* Hematology 2008; 13:119-127

There are several reports of the use of autologous transplantation both in relapsed disease and in first response. Five year OS is reported between 63% to 100% with a TRM of less than 10%.[226] The largest retrospective series of 158 patients with relapsed disease reports 5yr OS of 68% and 3 yr TRM 8%. The problem of course, is that this is not a curative procedure.[227]

As with other lymphoma subtypes, the use of MA conditioning in WM has resulted in unacceptable TRM. [169, 225] Reports of RIC-SCT in WM have demonstrated evidence of GVH activity and have a lower TRM. [173, 174] Kyriakou et al reported a retrospective analysis of 49 patients undergoing RIC with a 5 year OS of 64%, 5 year PFS of 49% and 3 year TRM of 23%. Whilst all of these had fludarabine based conditioning, over half were T cell depleted.[228] Similar retrospective studies using non TCD conditioning have reported similar outcomes.[229]

This study included 11 patients with WM with a five year OS of 53%. There were no relapses in surviving patients. Five patients died resulting in a cumulative 3 year NRM of 45%. Four of these were secondary to infection, and one to chronic GVHD. None of the patients had undergone a previous autologous SCT. Only 2 were in CR at the time of transplant and 5 were considered chemosensitive. Median number of treatment lines prior to transplant was 3. Seven patients had been treated with Rituximab. Three patients were over the age of 60 and all died of treatment related causes. Interestingly only 1 patient had an allograft after 2007. It is unclear whether this reflects a change in practice because of the high NRM in this subgroup or the rarity of finding a suitable transplant candidate with this diagnosis.

This data is too small to inform clinical practice with any validity. Whilst one of the potential advantages of RIC-SCT is that those who are older and with a worse performance status may benefit from it, it is clear that if our practice had been restricted to those under 60 years, outcomes in terms of survival and NRM would be superior. Bachanova et al suggests that RIC-SCT be reserved for younger patients with relapsed but chemosensitive disease, a high International Prognostic Scoring System for WM (IPSSWM) and a good performance status.[226] Clearly more data is needed to assist clinicians in how to identify who will benefit most from transplantation in this disease group.

5.5.5 Hodgkin lymphoma

Hodgkin lymphoma carries a favourable prognosis for the majority. However, 10-20% of patients are primary refractory and a similar percentage will relapse.[230, 231] Standard of care in both scenarios is salvage chemotherapy followed by an ASCT.[232] Relapse following ASCT carries a poor outlook with a median survival of 25 months. Early relapse within 6 months of ASCT holds an even bleaker outlook with a median survival of 15 months.[233] For those patients who relapse following ASCT, allogeneic stem cell transplant is recommended (table 16).

Despite this being a generally younger patient cohort, unacceptably high NRM associated with MA conditioning has resulted in the focus again lying with RIC transplantation.[234] Analysis of RIC-SCT in refractory/relapsed HL reports a PFS and OS of 20-40% and 40-64% at 2-4 years respectively. NRM is reported between 15-25% at 1-2 years.[235-240] These studies have demonstrated evidence of the GvH effect in HL and also suggest disease status and chemosensitivity impact upon outcomes.

This analysis included only 6 patients with HL, all under the age of 50. Three were primary refractory and the others had relapsed disease. The longest prior response period of these three patients was 7 months. Five had undergone a previous ASCT, 3 were considered chemosensitive and only 1 patient was in a CR at the time of transplant. Three patients died before 100 days from acute GVHD, TTP and an intracerebral bleed. There were no relapses. The NRM is clearly high, but patient numbers are very small.

RIC-SCT is considered standard of care for those with relapsed disease (table 16). However, outcomes are worse compared to other lymphoma subtypes. There has been suggestion that more intense conditioning may be required in order to allow disease control whilst the GvL effect is given time to occur. It has also been suggested that allograft should perhaps be considered earlier in the disease course or in those in whom the benefit of an ASCT is likely to be less, for example those who remain PET positive despite response.[241] Further studies are required in order to be able to answer these questions and guide our practice in where allograft is best placed.

5.5.6 Peripheral T cell lymphoma

T cell lymphomas are characterised by a worse prognosis than B cell lymphomas with a 5 year OS of 41%.[242] The BSBMT guidelines suggest that as with other lymphoproliferative disorders, ASCT is considered standard of care at relapse and allogeneic transplant is considered standard of care for those who relapse post ASCT or in those who are primary resistant (table 18).

Demonstration of disease control by virtue of DLI and withdrawal of immunosuppression again provides evidence of graft-vs-lymphoma in this subtype.[175, 176] OS and PFS following RIC-SCT are reported at 46% to 81% and 30%-64% respectively at 3 years. NRM is reported as 12-27%.[176, 243-247]

The small cohort of 6 patients in this study had a 5 year OS of 40%. Three patients died of treatment related causes; 2 of these were over the age of 60. One patient relapsed but was salvaged with DLI. The numbers are too small to draw any conclusions. However, what is interesting is the patient selection. Four out of the 5 patients had 1 line of treatment. They were all in CR with chemosensitive disease and no prior autograft. This suggests patients are being selected to consolidate their CR1 with an allograft that offers a curative potential rather than waiting until later in the disease course. Transplantation in CR1 is a óclinical optionô according to BSBMT and this may well be an appropriate strategy but larger numbers are required to provide more useful guidance on this (table 18).

CHAPTER SIX

Discussion

6.1 Introduction

Progress in our understanding and practice of haematopoietic stem cell transplantation has come a long way since its inception in the 1950s. Despite this, there is ever-increasing controversy within most haematological malignancies about the role and timing of transplantation. The more we understand, the more treatment options we have, the more questions are raised about exactly which patients should undergo SCT, when and with what conditioning regimen. Medical practice today is governed by óevidence-based medicineôand guidelines. However, in the field of SCT, guidelines are generic, with little quality evidence base available in many scenarios to help clinicians make decisions about transplantation in patients who fall into those controversial categories.

Haematopoietic stem cell transplantation at St Bartholomewôs Hospital demonstrates increasing activity year-on-year, in-line with national data reported by BSBMT. This rise is multifactorial. Increased early identification of factors that confer poor-risk in many haematological malignancies, use of reduced-intensity conditioning and availability of matched unrelated and alternative donors are some of the reasons behind the increasing number of patients proceeding to SCT.

I have looked at the outcomes of SCT in four different clinical scenarios to try and elucidate if local practice at St Bartholomewôs hospital can shed light into and inform practice in these areas of debate.

6.2 Autologous stem cell transplantation in myeloma

I evaluated the use of reduced-dose conditioning in ASCT using a MEL140 approach in patients with newly diagnosed myeloma with renal impairment, who were older than 65 years or with comorbidities felt to preclude them from the standard MEL200. Following these results, my hypothesis was rejected. The data demonstrated the use of MEL140 to be a safe and effective approach in this cohort of patients. Whilst accepting the limitations carried by this retrospective analysis and the smaller number of patients in the MEL140 arm, I would recommend that this is a viable approach in patients who would otherwise not be deemed suitable for ASCT. Although the MEL140 approach has not been directly compared to a non-transplant approach in this patient group, the equal outcomes in comparison to the MEL200 cohort infer that these patients are likely to be achieving an improved PFS and OS.

Prospective studies directly comparing MEL140 and MEL200 and comparing MEL140 versus chemotherapy alone would be helpful additions in evaluating the role of this approach.

For decades, patients with myeloma were treated with melphalan and prednisolone and had short life expectancies, with a median survival of 3 years.[248] During the 1990s, treatments changed and 4-6 months of vincristine, adriamycin and dexamethasone followed by high dose melphalan and ASCT became the standard of care in patients under the age of 65. This strategy improved survival and quality of life outcomes.[50, 249] Today, for the majority of patients, decisions regarding upfront myeloma treatment are focused upon whether the patient is considered an appropriate candidate for autologous transplantation. For those that are, ASCT remains the backbone of first line treatment.

Various strategies have been investigated to try and further improve ASCT outcomes. Attempts at purging the stem cell harvest to reduce contamination by malignant cells have had no beneficial effect on outcomes.[250, 251] Tandem autografts remain a matter of debate in terms of whether there is a survival benefit. Randomised studies have concluded that those patients that achieve less than a VGPR following their first transplant are the most likely to benefit from the tandem approach. However these studies have been criticised for not being applicable to patients being treated with todayøs induction regimens, and studies are ongoing.[252-254]

Since the landmark studies identified a survival benefit from ASCT, treatment in multiple myeloma has changed significantly and improvements in survival have been dramatic, with the greatest survival gains seen in those under 50 years of age.[255] These improvements are attributed to the arrival of novel agents including thalidomide, bortezomib and lenalidomide, amongst other factors, such as improved supportive care. These novel agents have all been demonstrated to have significantly better response rates than their historical counterparts, with significantly higher proportions of patients achieving CR.[256-263]

The importance of achieving a CR has been well described with improved depth of response correlating with improved overall survival.[82, 83, 264] In the pre-novel agent era, one of the rationales behind proceeding to ASCT was that the CR rate following induction treatment alone was only 5-10% and this could be increased by consolidation with high-dose therapy. However, the reported PFS and OS benefit associated with achievement of CR has yet to be demonstrated in many of the trials reporting the successes of novel agent combination therapies by virtue of their high CR rates. Only two have reported an improved PFS with novel therapy induction[262, 265]

Definitions for how CR is measured have evolved over time. More recently, it has been reported that those patients who are able to achieve an immunophenotypic CR have a significantly higher PFS compared to those with a 'traditional' CR or even stringent CR where the normalisation of the serum free light chain ratio is an additional requirement. Median time to evidence of recurrent disease is only 3 months if a non-immunophenotypic CR is achieved.[266, 267] Furthermore, it is not simply achieving a CR that is predictive of improved outcomes. Having a sustained CR for greater than 3 years unsurprisingly results in vastly improved OS outcomes.[268]

So why the discrepancy between the improved CR rates and lack of survival benefits with these novel agents? The median follow-up for most studies evaluating novel agents is less than four years. This time-frame is likely to be too short to identify any significant survival differences and this may be something that becomes more apparent with longer follow-up. Gene expression profiling has also demonstrated that the survival benefit seen with CR is not universal but is unique and critical to survival gains in those patients identified as high risk.[269]

There has been some suggestion that given the improved response rates to novel agents, that ASCT should be deferred until later in the disease's natural history. A meta-analysis of nine randomised trials demonstrated no survival differences according to the timing of the transplant.[270] Furthermore, there is some concern that such an approach, where patients receive consecutive treatments with combination chemotherapy, rather than high-dose melphalan up-front, may allow cytogenetic subclones to emerge that will make responsiveness to treatment increasingly difficult. This is most likely to be true for those with high risk cytogenetics at presentation.[271] Genomic studies comparing minimally treated and more heavily treated patients have demonstrated that those treated with multiple lines of therapy have a worse gene expression profile with a very poor outcome to high dose melphalan and ASCT, suggesting the evolution of drug refractory disease.[272]

The question has been pushed further with some questioning whether autologous transplantation has a place at all in the era of novel agents. Retrospective data comparing combination chemotherapy versus ASCT have demonstrated no differences in PFS or OS.[273, 274] It has been observed that those who undergo an ASCT upfront spend less time overall actually receiving therapy and consequently suffer fewer treatment-related symptoms and side-effects, resulting in an improved quality of life - a factor missing from evaluation in many studies.[59] However these studies were not randomised and had short follow-up. The only prospective trial to date has reported an increased PFS in the tandem ASCT arm. This is in comparison to the non-transplant arm where patients received six further cycles of melphalan, prednisolone and lenalidomide following induction with lenalidomide and dexamethasone, but survival data is awaited.[275]

To date, the treatment approach in myeloma has been relatively uniform and the only question traditionally asked at diagnosis is whether the patient is young enough and fit enough to undergo ASCT. However, it is increasingly clear that not all myeloma is the same. Our understanding of cytogenetic risk stratification in myeloma has increased significantly and there have been variable reports on bortezomib overcoming adverse cytogenetic risk.-[276-278] However, meta-analyses of patient cohorts with poor risk cytogenetics post ASCT is yielding discrepant results. Sonneveld et al reported that patients with 17p deletion who received PAD induction, ASCT and bortezomib maintenance had significantly improved PFS and OS compared to the VAD arm.[265] Whilst there was a significant improvement between the two treatment arms, the 17p deleted PAD arm did not equal the outcomes of those with non-adverse cytogenetics. Most other studies have observed that despite bortezomib-based induction, those with poor risk cytogenetics including t(4;14) and 17p deletion, have been identified to gain minimal benefit from ASCT.[102, 279, 280]

At the other extreme, there is a small cohort of patients who remain in CR over a decade following their ASCT and survival curves in two studies with long follow-up have both demonstrated a plateau with approximately 10% patients alive and disease-free between 11 and 14 years post ASCT.[84, 281] Perhaps these patients have been cured. Is there a unifying predictive factor in those with such impressive responses? Understanding which patients are most likely to have a sustained benefit would be extremely important in defining the role and utilisation of ASCT in the future.

At present, ASCT is not considered a curative procedure for the vast majority. Indeed in St Bartholomew's own results, which are comparable to reports in the literature, median PFS was between 20 months. Whilst the NRM is very low, it carries significant short-term morbidity. So can we say goodbye to ASCT in myeloma? Not yet. There is no convincing evidence-base to date to suggest that we have a better treatment strategy. Data on current novel agents and those on the horizon is promising but until longer follow up is able to demonstrate a survival benefit with or without ASCT, the door cannot yet be closed on transplantation.

Furthermore, there is more to contemplate than survival benefits alone. It has been clearly demonstrated that novel agents in combination result in better responses, but that this carries a higher risk of toxicities. We are already seeing that despite better survival outcomes, lenalidomide used as post ASCT maintenance carries a higher risk of secondary malignancies.[282] If the future of myeloma treatment is a series of successive combination therapies, there would need to be heavy consideration into the inevitable associated toxicities and the impact this would have on quality of life. By the same token, if the risk of toxicities, and mucositis in particular, were better understood then this too could play a role in patient selection and perhaps melphalan dosing. Work here is underway with identification of single nucleotide polymorphisms that seem to predispose some patients to more severe mucositis. However, this still remains some distance away from altering clinical practice.[283]

Ultimately the question is not - what is the correct dose of melphalan? or - is ASCT a thing of the past? but recognising that myeloma treatment, like most other malignancies, is not a one size fits all approach. Further understanding of myeloma tumour biology and gene expression profiling to allow the development of accurate methods of risk and likely response stratification are the key to being able to utilise the increasing armoury of novel drugs and transplantation to maximise benefits and minimise unnecessary toxicities.

6.3 The role of allogeneic transplantation in myeloma

The role of allogeneic transplantation in myeloma remains highly contentious. Guidelines vary from suggesting it should only be performed within a clinical trial because there is no evidence base to support its efficacy to the BSBMT guidelines which suggest that a sibling allogeneic transplant can be considered standard of care.[110, 284] This led to my hypothesis to examine whether the benefits of reduced relapsed in allogeneic transplantation in myeloma outweighed the risks of associated toxicities.

This discrepancy and lack of consensus is reflected in the numbers of transplants being performed. Whilst autologous transplantation in myeloma is the most common indication for a transplant, with over 4,500 being performed between 2006 and 2011 in the UK, myeloma is the least common indication for an allogeneic transplant with only 54 being performed in the UK over the same time period.[55] Low patient numbers add to the difficulty of interpreting data and making evidence-based treatment recommendations, but therefore the cohort of 35 patients analysed in this report from a single-centre is actually an impressive number when considering overall activity in this area and the majority of patients undergoing this approach in the UK do so at St. Bartholomewõs Hospital.

Allogeneic transplantation continues to draw interest because the utilisation of a disease-free donor cell graft and the immune-mediated graft-vs-myeloma effect drawn upon using reduced-intensity conditioning have the potential to achieve long-term cure in a disease which is otherwise considered incurable.

Our data produced PFS and OS outcomes in line with other studies in the literature, despite the patient cohort comprising both those being transplanted up-front and those at relapse. The TRM of 5% at 100 days and 11% at 3 years is exceptionally low. T depleted conditioning has already been recognised to have a detrimental impact on survival in myeloma, most likely due to the loss of the graft-vs-myeloma effect.[98] This analysis reinforces the use of the T replete conditioning regimen, comprised of fludarabine and cyclophosphamide, where both safety and efficacy are well-demonstrated. This conditioning platform has been reported to have a favourable toxicity profile in the context of other haematological malignancies.[111, 155, 156] However, the remarkably low TRM is even more relevant in myeloma where high TRM rates have been one of largest obstacles in the way of allogeneic transplantation.

So is allogeneic transplantation standard of care in myeloma? The answer currently has to be no and therefore my hypothesis must be rejected. There is no data either in this analysis or in the literature that categorically demonstrates a survival advantage utilising the allogeneic approach. However, there is a survival plateau with over 25% patients remaining in long term remission. It has been difficult to identify a specific patient cohort who will particularly benefit in this analysis. It is been demonstrated to be equally difficult to draw conclusions in a much larger EBMT registry-based analysis that evaluated the outcomes of different treatment pathways to transplant. Despite over 7000 patients in the analysis, and identification that the best survival outcomes are achieved by those who undergo a tandem auto-allo approach in first response (within 8 months of each other), there is no comment on statistical significance and no definitive conclusions about how best to utilise allogeneic transplantation.[285]

As NRM declines, the greatest obstacle continues to be progressive disease. Given the median time to death following relapse was 23 months, perhaps the role of allogeneic transplantation should be considered differently to other malignancies. Does progression post transplant make it a complete failure? Progression following an ASCT is virtually guaranteed but of course the difference is the toxicity profile of an ASCT is considered low enough to justify a life-prolonging but non-curative strategy. A RIC-SCT is also life-prolonging in the majority, especially if the NRM is as low as reported in this analysis.

Perhaps the challenge therefore extends to identify not only which patients are most likely to be cured but also those that may achieve prolonged remissions. This approach would only be justified if quality of life was reasonable, and it is well recognised that the morbidity associated with chronic GVHD may well compromise that.

In order to try and improve on the high disease progression rate post transplant, there is increasing research on post transplant maintenance therapies with novel agents to use either prophylactically or at relapse, in synchrony with DLI, or where DLI has failed. These have been recognised to have immunomodulatory effects that may be able to enhance the graft-vs-myeloma effects without aggravating GVHD. Results are encouraging, although in many cases, the duration of response remains limited and there are concerns about drug-related toxicities.[286-288]

A limitation of this analysis is the absence of cytogenetic data; this is recognised as increasingly important prognostically and is now recommended as a standard diagnostic test. Given that there is data to support the ability of allogeneic transplantation to overcome poor risk cytogenetics, this is a cohort of patients where due consideration should be given to performing an allogeneic transplant. [123, 289] It will be very interesting to evaluate their outcomes and identify whether this is a cohort where allogeneic transplantation has the power to change their fate.

6.4 The role of sequential allogeneic transplantation in refractory AML, relapsed AML and myelodysplasia

The sequential allogeneic transplant strategy in patients with refractory and relapsed AML, and MDS, allows patients with poor-risk disease to receive intensive chemotherapy and proceed directly into a T replete RIC allogeneic transplant. This theoretically enables patients to avoid the delays of getting to transplant because of difficulty achieving remission or chemotherapy-related complications and allows analysis on an ñintent to treat basisò, since most studies of transplant in these settings do not include the denominator of patients who receive salvage therapy but never make it to transplant. Given concern over rapid disease progression in these poor-risk patients, this approach also allows for less delay whilst awaiting the graft-vs-leukaemia effect. My hypothesis therefore was to consider whether this approach should be standard of care in patients with refractory and relapsed AML and high risk MDS. Of note, even with this approach, four of the enrolled patients did not proceed to transplant as planned, further demonstrating the difficulty of getting to transplant in this patient cohort.

The data from St Bartholomewôs reports an OS comparable with other sequential approaches reported in the literature, despite a very poor patient cohort in terms of cytogenetic remission, remission status and HCT-CI. There was a high NRM of 39% at 3 years for all patients. However, the prognosis for the majority of patients without transplant in this cohort is dismal and such a high NRM must be weighed against the alternative, which is inevitable death secondary to disease for the majority.

Despite this, there were some relatively clear findings to help inform patient selection for this strategy. Patients with relapsed AML, with a CR1 greater than 6 months, demonstrated a clear benefit. Their OS at 5 years was 41.7% which was significantly better than their predicted outcomes.[131] Whilst numbers were small, patients with MDS also seemed to have better survival outcomes than expected with no patients relapsing. Larger numbers are needed to confirm this observation, but it is in line with other MDS sequential outcomes and appears to be a very promising treatment strategy in these patients.[144] On the basis of these findings I would recommend this transplant strategy as a clinical option in these two groups of patients. Patients with refractory AML and those with high risk diagnostic cytogenetics had particularly poor outcomes and on the basis of this data, I would not recommend using the sequential approach in them. For this patient population less toxic treatment strategies or clinical trials are more appropriate.

6.5 The role of allogeneic transplantation in lymphoma

According to BSBMT guidelines, for patients with DLBCL or indolent lymphomas, allogeneic SCT is only standard of care if a patient has relapsed following an ASCT. In those patients at first relapse it is considered a ôclinical optionô and similarly patients with PTCL and MCL can be considered for an allogeneic SCT in first response as a ôclinical option.ô

The cohort in this study produced outcomes in line with the literature with a 5 year OS of 60%, PFS of 52% and NRM of 25%. With regard to which patients are most likely to benefit from allogeneic transplantation, recurrent themes emerged, firstly as a whole cohort analysis, and then again by histological subtypes. Based on this data, I would advocate the use of RIC-SCT in patients <60 years, with chemosensitive disease, who are in a CR and who have progressed greater that 12 months after their last treatment. If all these criteria are met, outcomes are likely to be most favourable. Those patients under the age of 40 have particularly good outcomes with OS >80% at 5 years and I would particularly recommend this treatment strategy in younger patients, keeping in mind other desirable criteria.

However, this not a tick-box exercise and there will be patients with poor-risk disease who do not meet these desired criteria. Whilst those with chemoresistant disease and less than a CR at the time of transplant have inferior survival outcomes, there is still a survival plateau for both these groups. Therefore clinicians and patients must have informed discussions in these scenarios about the potential risks and benefits of an allogeneic SCT.

Of course, histological subtype is important and transplant decisions and their timing are clearly dependent on the subtype. There is no evidence at present to answer the question regarding whether first relapses should be consolidated with autograft or allograft. St Bartholomewœ retrospective data reports similar 5 year OS with both strategies. This comparison is not based on equally matched patient populations, being taken from two independent data analyses. However, it does not suggest the strategy that is overwhelmingly most successful. Survival differences are more likely to emerge in the long-term, because the allograft cohort will produce a cohort of long term survivors through its immune-mediated graft-vs-lymphoma effect. Whilst there may also be a handful of long-term survivors in patients treated with ASCT,[204] this is not the expectation for those with low grade NHL.

The patients in this retrospective analysis are too heterogeneous and too small in number to make recommendations regarding RIC-SCT and its role in the various lymphoma treatment pathways. In DLBCL and follicular lymphoma, I would suggest that there should not be an óautopilotô response whereby patients in first relapse automatically proceed to salvage chemotherapy and an ASCT. In appropriate patients, there should at least be consideration of which consolidation strategy is most appropriate. This work supports pre-existing data that patients with FL who have previously undergone an ASCT have inferior outcomes post allograft.[207, 209] Therefore to maximise its potential benefits, performing it earlier in the disease may result in the best outcomes. Furthermore, in the Rituximab era, patients can now be identified who are less likely to benefit from an autograft at first relapse and these patients should be considered for allograft in first response.[197] More data is clearly needed to support this as a valid treatment pathway given the increased NRM associated with the allogeneic approach.

The data for MCL RIC-SCT is particularly encouraging with 5 year OS of 73% and NRM of only 13%. This analysis would suggest that patients with chemosensitive MCL, in CR, who are not heavily pre-treated are the most likely to benefit from the allogeneic approach. On the basis of small numbers in this analysis, I cannot recommend that RIC-SCT in first response should be standard of care, but certainly should remain as a clinical option. Larger numbers may change this recommendation in the future.

The remaining LPD subtypes are difficult to make specific recommendations about due to small numbers in this analysis and limited data in the literature with which to comment on the place of allogeneic transplantation. For those patients, consideration of the pros and cons on an individual basis will have to continue until more evidence becomes available on when allogeneic transplantation is best placed in these patients.

The treatment landscape in lymphoproliferative disorders is changing. Numerous new drugs have emerged across all histological subtypes including several new classes of molecularly targeted agents. These have demonstrated varying degrees of efficacy.[290, 291] Furthermore, genomic profiling is resulting in the identification of new targets and ongoing development of novel therapeutic strategies.

Idelalisib, an oral PI3K inhibitor, has been identified as an effective treatment strategy in those with relapsed or refractory low grade lymphoma.[292] The Bruton Kinase inhibitor Ibrutinib has demonstrated efficacy in mantle cell lymphoma.[293] Whilst these have all improved the treatment options for those with relapsed disease, response duration is finite and these are not considered curative options. However, further research may demonstrate they have benefit as pre or post transplant therapies.

Perhaps the only therapeutic development amongst the LPDs to impact on transplantation is Brentuximab vedotin (BV), an anti-CD30 antibody-drug conjugate. This has demonstrated significant activity in relapsed or refractory HL. Its use as salvage therapy and in particular as a bridge to transplant has been demonstrated. More recently, its favourable impact on post RIC outcomes is being reported with improved PFS compared to patients not treated with the drug pre-transplant.[294-296]

As follow-up periods are extended, and newer agents are utilised in different treatment settings, the role and timing of transplantation may well continue to evolve. To conclude, RIC-SCT is a viable and potentially curative treatment strategy. Some disease subtypes, namely DLBCL (predominantly transformed FL) and MCL appear to demonstrate particular benefit in this analysis. More data is required to know which patients should be selected for RIC-SCT, when this should happen and whether this should be earlier in the disease course, instead of an ASCT in selected patient cohorts. With the data currently available, consideration should be given to the patient's age, disease response, sensitivity and duration of their last treatment response when considering if a patient is an appropriate candidate for RIC-SCT and its likely success.

6.6 Where is allogeneic stem cell transplantation heading?

In the diseases I have discussed so far, recurrent themes emerge. There are many scenarios in malignant haematology where prognosis is extremely poor. Allogeneic stem cell transplantation is the only truly curative treatment strategy in these cases. There are two fundamental problems - firstly the associated toxicities, and secondly the significant rate of relapse that still occurs post transplant. If allogeneic transplantation could be administered without its associated toxicities and with more assurance of its curative potential, the fate of many would undoubtedly be different. Instead, at present, we are focused upon how to identify those whose disease confers such poor risk that the gamble of SCT is worth it. Whilst we deliberate over the relative merits of transplantation in individual cases, research is headed in differing directions in order to try and deal with these conflicts.

6.7 How can toxicity be reduced?

A major break-through here was the introduction of reduced intensity conditioning. This has clearly reduced but not eliminated the NRM and much work is still needed. Improved supportive care with better transfusion support, pre-emptive screening for CMV reactivation and fungal infections and better antimicrobials may all help. We now have scoring tools to help identify those at risk of particularly high NRM which enables clinicians to reconsider in those cases whether transplantation is actually the best treatment strategy.[297] The introduction of T cell deplete conditioning was also done with the aim of trying to eliminate the toxicities of T cell mediated GVHD.[39] The consequent realisation that reducing GVHD also reduced the beneficial GVL effect means that research now is focussed on trying to pull apart the two. Work is ongoing to try and differentiate the immune mechanisms by which these two processes occur in order to try and manipulate them differentially. However, whilst some steps are being made in understanding the different immune mechanisms involved, it will be some time before this has a significant impact on clinical practice.[298, 299]

6.8 How can post transplant disease relapse be reduced?

Post transplant strategies to reduce disease recurrence are predominantly focused upon manipulating and trying to enhance the GvL effect.

One strategy is to use vaccinations directed against tumour-associated antigens to ‘boost’ the GvL effect that is so crucial to long term remissions.[300] This strategy theoretically relies upon vaccination being administered in the early post transplant period to induce *in vivo* generation of large numbers of anti-tumour lymphocytes without induction of immune tolerance. The early post transplant period is felt to be an ideal time to introduce disease-antigen targeted vaccines to take advantage of the rapid lymphocyte proliferation that is occurring in this time period. This strategy can be boosted further by the use of adoptive transfer of vaccine primed lymphocytes. This has been performed successfully both from the patient and from healthy donors, although there are difficult ethical issues regarding vaccination of healthy donors. Further vaccination is then administered to the patient following chemotherapy and transplant to induce further stimulation of the GvL effect.[301-304] Work is ongoing with animal studies and early phase trials to try and develop this treatment strategy.

Other strategies have been to utilise maintenance therapies post transplant to try and sustain prolonged remissions. Whereas tyrosine kinase inhibitors (TKIs) are frequently used post transplant for patients with CML and Philadelphia positive ALL, efforts are now focused on the administration of drugs that can maximise the GvL effect. Most work has focused upon immunomodulatory drugs and hypomethylating drugs in myeloma, LPDs and myeloid disorders.[305, 306] Research is ongoing in this area and to date the results are variable with some concern about increased risks of acute GVHD with the use of lenalidomide.[307]

Work in the haplo-identical transplant field has generated more immunotherapeutic strategies to try and prevent relapse whilst minimising GVHD. NK cells originating from the donor have been shown to demonstrate alloreactivity against neoplastic cells that manifest themselves when KIR molecules on the surface of donorõs NK cells are not engaged by certain HLA class I molecules on the surface of hostsô cells.[308 309] There has been interesting data reporting that infusion of NK enriched cells can result in sustained disease control with no evidence of GVHD.[310, 311] This area of adoptive immunotherapy is again work in progress but if progress is sustained, may expand the benefits of GvL to patients who would not otherwise be considered fit for a transplant.[311]

6.9 Can patient stratification be improved?

6.9.1 Patient stratification in AML

As discussed previously, for the majority, the role of allogeneic transplantation in those with relapsed or refractory AML carries little debate. Even if remission can be achieved with salvage chemotherapy, the prognosis in this setting is so poor, that SCT is considered standard of care and the only potential way of achieving a durable remission.[133, 135]

For those with AML in first remission, more considered thought is required to determine who should undergo an allogeneic transplant. Acute myeloid leukaemia is the commonest disease indication for an allogeneic transplant.[55] The majority of patients with AML will achieve a remission following induction chemotherapy.[126] However, sustaining a remission is much more difficult. Fifty percent of patients under the age of 60 years will relapse within three years and in those over the age of 60, this rises to 85%.[312]

The decision of whether post-induction therapy should be consolidation chemotherapy alone or involve an allogeneic transplant is based upon the likelihood of relapse. Relapse in those patients with adverse risk is 70-90% if treated with chemotherapy alone or 30-50% if consolidated with a myeloablative transplant.[313] BSBMT recommendations from 2013 are that those patients with intermediate or adverse risk should have an allogeneic transplant in CR1. They define adverse risk as those with poor risk cytogenetics by MRC criteria, those with secondary or therapy-related AML and those who fail to achieve a CR with induction therapy. All eligible patients with relapsed AML should be considered for an allogeneic transplant.[284]

It is clear from clinical practice that these divisions of risk are not entirely predictive of outcome; there are patients with ógood riskô who have early relapses and those with ópoor riskô who demonstrate durable remissions despite not undergoing transplantation. However, setting aside patient fitness and donor availability, risk stratification remains the biggest determinant in making the decision to recommend a SCT. Our ability to do this accurately is incredibly important, given the significant risk of morbidity and mortality implicated in recommending a transplant. However our ability to stratify risk, whilst evolving, remains imperfect.

Identification of cytogenetic and molecular abnormalities are fundamental to making decisions about which patients in CR1 should have a SCT. Cytogenetic abnormalities are detected in approximately 55% of patients with AML and are divided into three prognostic groups.[314] Large meta-analyses have confirmed that those patients with intermediate and poor risk cytogenetics acquire benefit from an allogeneic transplant with an increased OS compared to those treated with chemotherapy alone. Those with good risk karyotype do not however demonstrate a survival benefit from SCT.[315, 316]

Forty to fifty percent of patients with AML have a normal karyotype and fall into the intermediate risk category. Despite the unifying label, this relatively large cohort of patients demonstrate significant variability in their survival outcomes. Consequently they are the subject of the greatest area of controversy regarding the identification of which patients should undergo a SCT in CR1.

Molecular genetics are helping to subdivide this category into more clinically relevant prognostic categories. In 2008, Schlenk et al reported the outcomes of 872 patients with normal karyotype in whom molecular genetic analyses were performed. They identified that those with mutations in NPM1 and CEBPA demonstrated improved outcomes and that SCT was particularly beneficial to patients with FLT3 ITD mutations who carried a worse prognosis.[317] Further studies have built upon this and there is now reasonable consensus that those with biallelic CEBPA mutations and NPM1 mutations carry good risk and should not have a SCT in CR1, unlike those with FLT3 ITD mutations.[318-321] This clinical practice, based upon molecular genetics, has not formally made its way into consensus guidelines, but there is little debate amongst transplanting clinicians that these results are fundamental in determining relapse risk in today's practice.

More recently, numerous other recurrent somatic mutations have been identified. It is clear from whole-genome sequencing that there is a complex interplay of genetic events which is likely to account for hetererogeneity in clinical outcome seen within AML.[322] Determining the clinical relevance of these mutations is difficult because it is likely that allele burden and the presence of molecular abnormalities in combination impact upon outcome. However, strides forward are occurring and there is now evidence that more extensive mutational analysis may allow better discrimination of those considered intermediate risk by standard cytogenetic criteria.[323] Integrated genetic profiling may in time assist in identifying those most likely to benefit from SCT or indeed from novel targeted therapies.

Whilst much of the focus is on how to pull apart those with normal karyotype, it is important to remember that even those with 'favourable risk' have no guarantees of long-term remission. The adverse prognosis of KIT mutations in those with favourable cytogenetics has already identified this as a separate cohort that may benefit from c-KIT inhibitors or even SCT.[324]

The commonest cause of transplant failure in AML remains relapsed disease. The presence of MRD positivity in those considered to be in CR by conventional criteria prior to SCT has been identified as a predictor of increased relapse and reduced survival post transplant.[325] Similarly those patients that took more than one cycle of chemotherapy to achieve CR1 have a significantly worse prognosis post transplant, independent of all other factors.[326] Both of these factors are undoubtedly a reflection of chemo-resistant disease with the data suggesting that SCT is unable to overcome the inherent treatment resistance in these patients. Further work is needed to understand the role of MRD in determining the role and timing of transplant and whether those with chemo-resistance may benefit from a non-transplant strategy.

The risk carried by the disease is only half of the equation. The reality is that if allogeneic SCT carried no associated NRM, it would be recommended as a consolidation strategy in all patients with AML. It is not that it is ineffective in those with ‘good risk' disease, merely that the associated NRM is too high to overcome the potential survival benefit. Consequently, only those with the highest risk of relapse have the potential to increase their survival despite the NRM risk.[327]

In the same way that disease risk stratification is needed, stratification of NRM risk is also required. Consideration of the risk of age, comorbidity and likelihood of GVHD are essential when making decisions regarding transplantation. Better supportive care, better HLA-matching and tools to help identify appropriate patients will all help to reduce NRM.[297, 328-330] If patients are selected with care, those that undergo the procedure are more likely to achieve the potentially curative benefits.

6.9.2 Patient stratification in myelodysplasia

Allogeneic SCT is the only curative treatment strategy available to those with myelodysplasia. Most current practice is based upon the recommendation that those with low risk disease (low or INT-1) based upon the IPSS do not gain benefit from SCT, whereas those with INT-2 or high risk disease should proceed to allogeneic stem cell transplant if there is an available donor.[141]

There has been concern that the IPSS score is only validated as a prognostic tool at diagnosis and that it excluded those with Chronic Myelomonocytic Leukaemia and treatment-related MDS. It also does not take into account the impact of age or comorbidities and was validated prior to the introduction of hypomethylating agents into the treatment of MDS.[331] However the same outcomes and recommendations have been made using the IPSS with SCT compared with best supportive care or hypomethylating drugs. The WPSS score has been demonstrated to be a better tool with which to estimate the timing of SCT.[332, 333]

It is well recognised that those MDS patients with less disease burden prior to SCT have reduced relapse post transplant. It might be expected that the introduction of the less intensive azacytidine might result in better outcomes compared to intensive induction therapies. The results of two retrospective studies have failed to demonstrate a difference in post-transplant outcomes, although prospective studies are underway to evaluate this further.[334, 335]

Like in AML, the factor most likely to impact upon the role of SCT is improved risk stratification. The impact of monosomal karyotype in MDS continues to cause controversy but it has been reported to have a 2 year OS of 6% post SCT.[336-338] If replicated, this suggests that allogeneic transplant is not an appropriate strategy with which to manage this sub-group. Significant progress has been made in identifying a multitude of point mutations implicated in MDS. There has also been demonstration that detection of these may allow the identification of cohorts of patients with a worse prognosis than is predicted by current scoring methods.[339, 340] Further studies will then be required to identify whether this poor prognosis can be overcome by SCT.

6.10 Can allogeneic transplantation be avoided altogether?

6.10.1 Chimeric antigen receptor T cells

Advances in gene therapy have been progressing steadily over the past decade with the development of chimeric antigen receptors (CARs). These are fusion proteins incorporating both antigen-binding and T-cell activation domains. [341] Autologous T cells are genetically modified to express CARs and any cell surface molecule can be targeted. Most work to date has been done with anti-CD19 CAR T cells which recognise and kill CD19+ target cells. CD19 is expressed on most malignant B cells and therefore these have been used with good clinical effect in a variety of B cell malignancies including ALL, indolent NHL, CLL and DLBCL.[342-346]

These promising results are leading to further research to identify effective surface molecules that can be targeted for other malignancies such as AML and myeloma.[347 348] Whilst there is a risk of cytokine release syndrome and B cell aplasia, excellent responses have been demonstrated in patients with refractory disease. At present CARs are utilised to achieve response prior to transplant or in those who have relapsed post transplant. However, if their efficacy is demonstrated to be sustained, it is possible this treatment could avoid the need for allogeneic transplantation altogether

6.10.2 Targeted drug therapies

The development of targeted drug therapy that results in high response rates with prolonged remission is another treatment strategy to try and bypass the need for allogeneic transplantation and its associated toxicities all altogether. I will now discuss how targeted therapies have changed the role of transplantation in CML, probably the best demonstration of success in this area, and CLL below.

6.10.2.1 Targeted drug therapies in chronic myeloid leukaemia

The story of CML is a remarkable tale of the evolution of stem cell transplantation in haematological malignancy.

In 1974, the first successful allogeneic transplants in CML were reported in 4 patients with syngeneic donors who remained in remission and philadelphia chromosome negative 2 years post transplant.[349] IBMTR data reported ongoing successes, utilising HLA matched sibling donors, with clear demonstration of a survival plateau between 14-19 months post transplant. Their report also clearly demonstrated that those transplanted in chronic phase had significantly better outcomes than those transplanted either in accelerated or chronic phase, both in terms of survival and disease recurrence.[350]

With the development of unrelated donor registries, allogeneic transplantation grew and in 2000, CML, alongside AML, were the most common indications for an allogeneic transplant.[351] Recognition of the T cell mediated GvL effect drove the development of reduced-intensity conditioning in many haematological malignancies and just as plans were underway to compare myeloablative and RIC platforms in clinical trials, the game-changing tyrosine kinase inhibitor, Imatinib, arrived on the scene.[352]

Imatinib has revolutionised CML treatment over the last decade. Follow-up data has reported that 8 year OS and PFS are 85% and 92% respectively when used first line in chronic phase.[353] Even before formal follow-up data was available, the use of SCT in CML was already on the decline.[354] Today, the demonstration of such successful results using an oral tablet with reported minimal toxicity has resulted in SCT no longer being recommended first line in patients in chronic phase.[355]

However, 15-25% of patients will fail or be intolerant to Imatinib. SCT remains the treatment of choice in patients who present in or develop accelerated (AP) or blast phase (BP). One of the consequences of TKI therapy, is that more patients will now be transplant candidates in AP or BP when it is well documented that survival and relapse outcomes are significantly worse.[350] Transplantation in BP is only successful in 10% of cases.[356]

Three year OS data in the Imatinib era is reported at between 72-74% following SCT with no negative impact on outcomes observed by prior TKI treatment.[356-358] EBMT data has observed improving SCT outcomes over time.[354] Post transplant strategies that allow MRD monitoring and consequent utilisation of the synergy between TKIs and DLI may improve outcomes further.[359, 360]

Treatment with TKIs started in 2001 ï we now have 14 years of follow-up with 8 years formally reported in the literature. Over that time, compliance issues, intolerance and development of mutations are challenges that have been added to the equation. For a patient diagnosed in their 20s, there is no real data in the TKI era about his prospect of reaching old-age. Attempts to discontinue Imatinib following prolonged and convincing molecular remissions have demonstrated that approximately half of those patients will relapse within 6 months.[361] Quality of life (QoL) assessments have revealed that many patients on Imatinib have a reduction in QoL due to drug side effects and one study reported a significantly superior QoL in those who had a SCT compared to those on Imatinib.[362, 363]

So whilst other diseases look on in hope, there is still a clear, albeit modified, role for SCT in CML and still much work to be done on the quest to make CML a disease where SCT truly is a thing of the past. Researchers and clinicians managing other haematological malignancies are however still hoping to find their ÷Imatinib-equivalentô in order to avoid the ongoing morbidity and mortality associated with allogeneic stem cell transplantation.

6.10.2.2 Targeted drug therapies in chronic lymphocytic leukaemia

CLL is the commonest adult leukaemia. In 2007, EBMT presented a consensus statement whereby those patients with poor-risk CLL should be considered for an allogeneic stem cell transplant. Poor risk included patients who did not respond to or relapsed within 12 months of treatment with a purine analogue, those who relapsed within 24 months following an initial response to purine analogues or an ASCT or those carrying p53 abnormalities.[364]

The introduction of RIC-SCT was of particular importance in CLL because the median age at diagnosis is 70 years, meaning that in the era of myeloablative conditioning, SCT was not an option for many.[365]

Over the last few years, there has been an explosion of activity in the development of new drugs in CLL. Brutonøs tyrosine kinase (BTK) holds a central role in the activation of several constitutively active pathways and has therefore become an attractive target to try and inhibit therapeutically. Ibrutinib is an oral, irreversible BTK inhibitor and appears to be the most promising development in the recent spate of new therapeutic advances. With a 26 month PFS and OS of 75% and 83% respectively, its outcomes are significantly better than other single agents are able to achieve in a cohort of relapsed/ refractory patients with a median of 4 treatment lines and an otherwise profoundly poor prognosis.[366, 367] Its toxicity profile is also very favourable with minimal grade 3 or 4 events. Furthermore, its outcomes in those with refractory CLL with 17p deletion, although not equal to the remainder of the cohort, are again superior to outcomes with other treatments.[366]

PI3K is another essential kinase involved in activation of pathways of importance in cellular proliferation, survival and differentiation. Idelalisib is an oral PI3K inhibitor which has also demonstrated favourable outcomes, albeit with a shorter duration of follow-up reported to date. In combination with Rituximab, a 92% OS at 12 months has been reported. It too has demonstrated favourable results in those with 17p deletion.[368]

Other studies are underway including BCL2 inhibitors and the development of chimeric antigen receptor (CAR) T cells.[344, 369] The arrival of these novel agents has resulted in those with classically poor-risk CLL, especially those with 17p deletion, having more treatment options available. The follow-up data on these drugs remains short but their impressive outcomes have the potential to change the standards with question-marks already hanging over the place of SCT in our current treatment algorithm.

Despite excellent response rates, the proportion of patients achieving a CR with Ibrutinib or Idelalisib monotherapy is low. This suggests that SCT will continue to have a role, either at the point of relapse or in a defined poor-risk cohort as consolidation. [370]

These are exciting times for CLL. Oral agents that allow patients of all ages to achieve excellent clinical responses whilst avoiding the toxicities of chemotherapy is a huge stride forwards and will inevitably change our current practices. However, longer follow-up data, and further prospective trials comparing these new drugs either alone or followed by SCT in comparison to our current treatment practices are needed in order to guide clinicians on how best to utilise these novel agents in conjunction with SCT.

6.11 Conclusion

I have evaluated four areas of stem cell transplantation practice at St Bartholomew's Hospital and in conjunction with a review of the literature, I have made recommendations regarding ongoing practice in these controversial areas. The spectrum of controversy is however much wider than the areas I have evaluated in this research.

Haematopoietic stem cell practice is continuously evolving and the future of SCT is unclear. Will it evolve into a highly refined treatment with minimal toxicity and high cure rates or will it become yesterday's news, trumped by novel therapies with minimal toxicities? Research into the genomics of haematological malignancies is gaining pace, and undoubtedly, being able to refine disease-stratification across all malignancies is fundamental to personalising their approach to treatment as a whole, including the role of transplantation. However, progress in the development of molecularly targeted drugs, immunomodulatory and gene therapies may significantly change the way SCT is incorporated into treatment pathways in the future.

Appendix 1: Sequential trial ethics approval

National Research Ethics Service

East London and the City Research Ethics Committee 1
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Telephone:
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23 July 2007

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Senior Lecturer and Honorary Consultant in Medical Oncology
Barts and the London NHS Trust
Pathology Department, Level One
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Dear Dr Cavenagh

Full title of study:	**A Phase II Trial of Sequential treatment with Cytoreductive therapy and Reduced Intensity Conditioning Allogeneic Stem Cell Transplantation for Relapsed/ Refractory Acute Myeloid Leukemia, High Risk Myelodysplasia,or other High Risk Myeloid Malignancies**
REC reference number:	**07/Q0603/65**
Protocol number:	**Protocol Ref N/A**
EudraCT number:	**2007-000806-64**

Thank you for your letter of 21 June 2007, responding to the Committee's request for further information on the above research.

The further information was considered at the meeting of the Sub-Committee of the REC held on 18 July 2007. A list of the members who were present at the meeting is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document and subject to receiving a final CTA approval when available. You are advised to study the conditions carefully.

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	1	04 May 2007
Investigator CV		04 May 2007
Covering Letter		04 May 2007
Covering Letter	2	21 June 2007
Peer Review		12 February 2007
Participant Information Sheet: Patient Information Sheet	1	29 December 2006
Participant Information Sheet	2	21 June 2007
Participant Consent Form: Consent Form	1	02 February 2007
Response to Request for Further Information	1	21 June 2007
Applicant's checklist	1	04 May 2007
GP Letter	1	01 February 2007
MHRA request for authorisation		04 May 2007
Protocol	1	12 January 2007
Sponsorship Approval Letter		10 April 2007
Statement of Indemnity Arrangements		10 April 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Feedback on the application process

Now that you have completed the application process you are invited to give your view of the service you received from the National Research Ethics Service. If you wish to make your views known please use the feedback form available on the NRES website at:

https://www.nresform.org.uk/AppForm/Modules/Feedback/EthicalReview.aspx

Appendix 2: Sequential study protocol

Barts and The London School of Medicine and Dentistry

Barts and The London NHS Trust

Protocol Version 2.0, Dated 1st August 2011

TITLE OF PROTOCOL:

A Phase II Trial of Sequential treatment with Cytoreductive therapy and Reduced Intensity Conditioning Allogeneic Stem Cell Transplantation for Relapsed/ Refractory Acute Myeloid Leukaemia, High Risk Myelodysplasia, or other High Risk Myeloid Malignancies

Sponsor Protocol Number: 004973
EudraCT Number: 2007-000806-64

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Sequential Allo Protocol Version 2.0 1st August 2011 Page 1 of 25

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1 PROTOCOL SUMMARY

Eligible patients with Refractory or Relapsed Acute Myeloid Leukaemia (AML), High Risk Myelodysplasia (MDS), or other high risk Myeloid Malignancy, will receive treatment with a High dose Ara C containing regimen (or other cytoreductive therapy as clinically appropriate) followed by a three day rest period. Subsequently, they will be conditioned with Fludarabine 25mg/m2 for five days (5 doses) and Cyclophosphamide 1g/m2 for two days (2 doses) and receive Haemopoietic Stem Cell Transplantation from a sibling or unrelated donor. Graft versus Host Disease (GVHD) prophylaxis will be in the form of Ciclosporin and Methotrexate. Donor Lymphocyte Infusion (DLI) in the presence of Mixed Chimerism or evidence of persistent disease will be considered at Day 120 depending on the presence of GVHD or concurrent infections.

2 BACKGROUND AND RATIONALE FOR PROPOSED STUDY

Outcomes for patients with refractory or relapsed AML are generally extremely poor (1). Indeed, for patients whose AML has demonstrated refractoriness to conventional therapy, which can be defined as primary induction failure, a first remission duration of less than six months, second or greater relapse or refractory relapse, outcomes are particularly dismal. For this group of patients, the probability of achieving a remission with high dose Ara-C containing regimens is approximately 10-15% with one year survival of less than 10% (2). Indeed, the median survival in this setting is less than four months. Furthermore, for any patient with relapsed disease outwith the definition of 'refractory disease' stated above, less than 50% achieve a second remission and overall survival ranges from 3-12 months (3-5).

Nevertheless, there is heterogeneity in outcomes with the major factors influencing outcome being the duration of first remission and cytogenetic risk category. The HOVON group have generated a prognostic index for AML (excluding APML) in first relapse derived from a scoring system incorporating duration of first remission, cytogenetics at diagnosis, age at relapse and whether the patient had undergone prior stem cell transplantation (6). The derived score allows patients to be allocated to one of three prognostic groups. Five year survival for patients in the favourable, intermediate and poor risk groups are 46%, 18% and 4% respectively. Importantly, only 8% of patients fell in to the good risk group, with 25% and 68% in the intermediate and poor risk groups respectively.

Therefore, one can conclude that outcomes for patients with relapsed AML are extremely poor, with the exception of the small minority who have relatively favourable features. It is recognised that AlloSCT provides the best hope for prolonged DFS in this group of patients (7-9). In distinction to chemotherapy, AlloSCT exerts its anti-leukaemic effects through allogeneic immunological mechanisms. In the fully HLA-matched setting, differences in minor histocompatibility antigens between donor and recipient result in the generation of alloreactive T-cells that mediate so-called graft versus host (GVH) along with graft versus leukaemia effects (GVL). This alloreactivity significantly reduces relapse risk and can result in durable leukaemia-free survival. Conventional, ablative AlloSCT combines high dose, anti-leukaemic chemoradiotherapy with subsequent infusion of allogeneic stem cells and T-cells which repopulate the haemopoietic system and exert GVH/GVL effects respectively. Utilising ablative AlloSCT in second remission, 30-40% durable leukaemia-free survival (LFS) can be achieved (10) and on this basis the BCSH recommend AlloSCT for younger patients in second remission (11).

However, there are a number of factors that mitigate against an attempt to achieve a second remission prior to AlloSCT. Firstly, the probability of achieving a second remission is low, indeed, less than 50% of patients overall will do so (6). Secondly, reinduction therapy is associated with considerable morbidity and mortality in its own right (6). For those patients with primary induction failure or remission duration less than six months, the mortality of attempted reinduction approaches 25% (8,12,13). Thirdly, reinduction therapy will result in variable durations of profound cytopenia during which time there is a considerable risk of opportunistic infections, particularly invasive fungal infection, which may contraindicate, or increase the risks of, any subsequent AlloSCT (14). Fourthly, the hospital stay required for reinduction is costly and utilises resources. With all of these disadvantages in mind, it may well be preferable to proceed directly to AlloSCT at the time of relapse, a strategy found to be feasible and effective by the Seattle group (15). Similarly, it is feasible to proceed directly to AlloSCT for patients with primary induction failure since long term leukaemia free survival of 20-30% at best can be achieved in this setting (16).

All of the above statements have been used to justify proceeding directly to ablative AlloSCT at the time of primary induction failure or relapse. Although there is clear evidence that such a strategy can result in long term DFS, ablative transplantation is associated with significant toxicity and mortality and these risks rise exponentially with advancing age. Indeed, in a large retrospective analysis by the IBMTR, two year NRM reached 90% in patients with advanced leukaemia over the age of 45 years (17). A major breakthrough in the last 5-10 years has been the development of so-called reduced intensity transplants (RIC-Allos). This strategy permits application of the profound immunologic anti-leukaemic effect of allogeneic transplantation in older individuals or those with comorbidities. Such RIC-Allos have been shown to be feasible for a broad range of haematological malignancies and have been applied to poor risk myeloid leukaemia and myelodysplasia. A number of groups, including ours, have reported encouraging results with this strategy (18-20). In our study, a low intensity conditioning regimen incorporating fludarabine and cyclophosphamide, along with cyclosporin and methotrexate as GVHD prophylaxis, was used. We observed an encouraging low transplant-related mortality rate of 4% with grade I-II acute GVHD at 16% whilst no grade III-IV acute GVHD occurred. No cytomegalovirus (CMV) disease was seen with only 12% CMV reactivation. With a median follow-up of 3.5 years, encouraging estimated 4 year overall and event-free survivals of 55% and 40% respectively were observed (20). Generally speaking, such RIC-Allos have been performed when patients with AML are in remission since the pure, allogeneic immunological GVL effect is relatively slow in onset. Thus, patients who have active disease at the time of RIC-Allo are felt to be at high risk of early relapse as the kinetics of AML proliferation will outpace the onset of effective GVL.

With this background, a collaborative German group has studied a strategy of sequential chemotherapy and RIC-Allo with the aim of increasing the safety of AlloSCT and yet maintaining the antileukaemic efficacy of allogeneic transplantation (21). Patients with refractory or relapsed AML initially received cytoreductive therapy with a high dose Ara-C containing regimen with the aim of reducing the leukaemic cell burden prior to AlloSCT using a matched related or unrelated donor. After a three day rest, patients proceeded directly to a RIC-Allo. A subset of patients also received prophylactic donor lymphocyte infusions (DLI) 30 days after cessation of immunosuppression in the absence of active GVHD or infection.

A total of 103 patients were treated (37 primary induction failure, 53 in early relapse, 8 with refractory and 5 with second relapse), with a median age of 52 years (range 18-68 years). 99/103 had active disease at the time of treatment and the cohort had significant comorbidities as evidenced by 58% having a Charlson comorbidity score (CCI) score of 1 or more. OS at 1, 2 and 4 years was 54%, 40% and 32%. Respective LFS survival was 47%, 37% and 30%. The one year NRM was 17.2%. These are highly encouraging results, both in terms of treatment related mortality and long-term disease control. The outcomes compare favourably with data derived from large cohorts of relapsed AML patients (such as the HOVON analysis) and also with data from patients treated with ablative AlloSCT. We therefore propose to study this approach further in patients with refractory and relapsed AML, as well as in patients with poor-risk myelodysplasia (MDS) (see below), to determine whether we can confirm these favourable outcomes. We plan to use a similar high dose Ara-C containing regimen as is currently the standard for salvage therapy in our centre for younger patients. Likewise, we plan to use a standard-dose Ara-C containing regimen for older patients (or those with renal impairment) since the risks of Ara-C toxicities (especially cerebellar) are greatly elevated in older patients or those with renal impairment. We further propose to use our current RIC-Allo conditioning schedule which has been demonstrated to be safe and effective.

As already mentioned, we also propose to utilise this same schedule for patients with poor-risk MDS. Again, allogeneic transplantation remains the only potentially curative treatment for MDS (22). Outcomes for patients remain poor (see Table).

IPSS risk group	OS < 60 yrs	OS > 60 yrs
INT-1	5.2 yrs	2.7 yrs
INT-2	1.8 yrs	1.1 yrs
High	0.3 yrs	0.5 yrs

In the light of these poor outcomes with current therapies, The BCSH guidelines for the management of MDS suggest that AlloSCT should be considered for all patients with INT-1/INT-2/High risk MDS (22). With a sibling donor, it is suggested that patients less than fifty years of age should undergo an ablative transplant whereas those older than fifty should receive a RICAllo. The same recommendations are made for patients with an unrelated donor, except that the age cut-off is 40 years. Furthermore, these guidelines recommend the application of intensive chemotherapy (induction and consolidation) prior to transplant in patients with INT-2/High risk MDS. It is suggested that only those patients in CR/good PR should proceed to transplant. It is recognised that the probability of achieving CR in these circumstances is approximately 40-50% and that the TRM associated with ablative AlloSCT is 40% (22). Therefore, if utilising an ablative AlloSCT approach, only 16-20% of patients initially treated with a view to proceeding to transplantation will actually receive the transplant and not succumb to treatment-related complications. Of these, it is reasonable to suggest that 50% will subsequently relapse. In other words, for a patient with poor-risk MDS treated with a transplant strategy, there is approximately only a 10% probability of long-term disease control. We feel, therefore, that alternative approaches should be explored in an attempt to improve these poor outcomes. In particular, in the light of the highly encouraging results from the Munich group in patients with poor-risk AML, we propose to utilise this approach for patients with MDS also.

A contentious issue in the treatment of MDS patients is the requirement for remission induction therapy prior to transplant. The BCSH guidelines group recommend such therapy although there are no prospective trial data to justify this approach. The major rationale for induction chemotherapy is the perception that patients who are in remission at the time of transplant will have a lower risk of relapse. However, there are powerful counter arguments to consider. Firstly, as already mentioned, only 40-50% of patients will achieve remission, meaning that half of eligible patients will be exposed to the toxicity of intensive chemotherapy without proceeding to transplant. Secondly, 15-20% of patients will die from complications of attempted remission induction (23). Thirdly, surviving patients in remission may well have developed complications following chemotherapy, such as invasive fungal infection, which contraindicate transplant or increase its risks (14). As previously stated, there are no prospective trial data to formally address the issue of the benefit or not of attempted remission induction prior to transplant. Some retrospective studies have been performed such as by the Seattle group (24). All transplanted patients had AML secondary to MDS or therapy-related AML. Those transplanted 'upfront' had a 24% five-year DFS, whereas those transplanted following chemotherapy, had a 15% DFS. With all of these issues in mind, we therefore propose to test the Munich strategy in patients with MDS. A particular attraction in this setting is the sequential nature of the schedule, combining anti-myelodysplastic chemotherapy with allogeneic transplantation. All patients eligible for transplant will receive it and they will therefore be spared two 4-6 week hospital admissions for remission/consolidation therapy.

3. OBJECTIVES

3.1 General Statement

The purpose of this study is to:

a) Evaluate the efficacy of this approach in inducing durable remissions in this high risk group of patients
b) Assess the time to engraftment and overall time of cytopaenias.
c) Assess the overall duration of in-hospital stay.
d) Evaluate the role of Donor Lymphocyte Infusion (DLI) in the post transplant period

3.2 Endpoints

3.2.1 Primary endpoints

1) Overall Survival (OS) at 1,2 and 4 years.

3.2.2 Secondary endpoints

1) Event Free Survival (EFS) at 1, 2 and 4 years
2) Treatment Related Mortality (TRM) at d100, 1 and 2 years and cause of mortality.
3) Incidence and Grade of Acute Graft versus Host Disease (GVHD)
4) Incidence and Grade of Chronic GVHD.
5) Time to Engraftment*
6) Full Split Chimerism at Days 30, 60, 100 and 1 year.
7) Request for DLI (Mixed Chimerism vs. Disease persistence)
8) Incidence of Opportunistic Infections
9) Duration of Hospitalisation

*Date of Neutrophil Engraftment is defined as the first of two consecutive days with a neutrophil count exceeding 500/µL. Date of platelet recovery is considered the first of three consecutive days with an unsupported platelet count exceeding 20 x 10^9 / L

4.0 DESIGN

4.1 General

This is an open label phase II study

Approximately 90 – 95 patients will be entered into this study.

The study commenced recruitment in April 2007 and will have a duration of 48 – 60 months. It is expected that the accrual rate for the study will be approximately 20 patients per year.

4.2 Population

All eligible patients with relapsed/refractory AML, High Risk MDS or other high risk myeloid malignancy who are not candidates for ablative bone marrow transplantation but are deemed suitable for combination chemotherapy and Reduced Intensity Conditioning HCT will be offered entry into the study.

4.3 Investigational Medicinal Products

For the purposes of this study (and in accordance with the CTA), the following will be considered as IMPs:

- Cytarabine
- Daunorubicin
- Cyclophosphamide
- Fludarabine
- Methotrexate

4.4 Schedule of Administration

Low Dose Cytarabine(10mg/m2 sc bd for 14 days to be repeated at 28 day intervals if necessary) can be administered at the Investigator's discretion for disease control in patients with rapidly progressive disease when conditioning has to be delayed (e.g awaiting unrelated donor clearance).

D-15	Daunorubicin 45mg/m2 OD IV Cytarabine 1.5g/m2 (1 dose-pm) IV
D-14	Daunorubicin 45mg/m2 OD IV Cytarabine 1.5g/m2 BD IV
D-13	Daunorubicin 45mg/m2 OD IV Cytarabine 1.5g/m2 BD IV
D-12	Cytarabine 1.5g/m2 BD IV
D-11	Cytarabine 1.5g/m2 BD IV

D-10	Cytarabine 1.5g/m2 BD IV
D-9	Cytarabine 1.5g/m2 (1 dose-am) IV
D-8	Rest Day
D-7	Rest Day
D-6	Fludarabine 25 mg/m^2 i.v. OD
D-5	Fludarabine 25 mg/m^2 i.v. OD
D-4	Fludarabine 25 mg/m^2 i.v OD
D-3	Fludarabine 25 mg/m^2 IV Mesna 400mg/m^2 IV in 1 litre N/saline over 1 hour Cyclophosphamide 1 g/m^2 IV in 500ml N/saline Mesna 400mg/m^2 IV in 1 litre N/saline over 1 hour Mesna 800mg/m^2 PO at 6 hours post Cyclophosphamide
D-2	Fludarabine 25 mg/m^2 IV Mesna 400mg/m^2 IV in 1 litre N/saline over 1 hour Cyclophosphamide 1 g/m^2 IV in 500ml N/saline Mesna 400mg/m^2 IV in 1 litre N/saline over 1 hour Mesna 800mg/m2 PO at 6 hours post Cyclophosphamide Load Ciclosporine 5 mg/kg i.v. od
D-1	Rest Day Reduce Ciclosporin to 3mg/kg i.v. (Maintain trough CsA levels between 150 – 300 ng/ml)
D 0	Allogeneic PBSC infusion.
D+1	Methotrexate 5mg/m2 IV OD
D+3	Methotrexate 5mg/m2 IV OD
D+6	Methotrexate 5mg/m2 IV OD

Ciclosporin to be tailed off between D60 – 90 as clinically indicated.

DLI to be considered 30 days after discontinuation of CiclosporinCiclosporin in the presence of Mixed Chimerism or evidence of persistent disease.

DLI schedule for Matched Sibling Allografts: 1 x 10^7 CD3+cells/kg increasing, in the absence of GVHD, at 4-6 weekly intervals up to three times to 5x 10^7 CD3+ cells/kg and 1 x 10^8 CD3+ cells/kg.

DLI schedule for Matched Unrelated Donor Allografts: 1x 10^6 CD3+cells/kg increasing, in the absence of GVHD, at 4-6 weekly intervals up to three times to 5x10^6 CD3+ cells/kg and 1 x 10^7 CD3+ cells/kg.

4.5 Dose Modifications

- It is the Investigator's clinical decision to use alternative cytoreductive regimens e.g Cytarabine1.5g/m2 IV BD on D 1,3,5 (total of 6 doses) or conventional (non HDAC) AML Induction chemotherapy when clinically indicated.

- The final dose of Methotrexate on Days +1, +3 and +6 will depend on the degree of mucositis and biochemistry results according to the Standard of Care of the Unit.

- Dose modifications to IMPs may also be made in accordance with local policies for hepatic and renal impairment

4.6 Concurrent Medication and Treatment

Patients will be receiving the Standard of Care of our Unit for Allograft recipients.

4.7 Schedule of Events

PARAMETER	BASELINE		DURING TREATMENT	AFTER TREATMENT		
	Within 4 Weeks	Within 1 Week		D +30	D +60	D +100
Informed Consent	X					
Demographic Data	X					
Medical History Baseline Conditions	X					
Prior Diagnosis / Treatment	X					
Height *		X				
Weight *		X				
BSA *		X				
ECOG performance score *		X				
Virology of Donor and Recipient	X					
FBC, Chemistry *		X	X (1)	X	X	X
CMV PCR (2) *			X	X	X	X
Ciclosporin level (2) *			X	X	X	X
Serum or urine HCG (women) *		X				
Lung Function Tests *	X					
Echoardiogram *	X					
Bone Marrow Aspiration/ Flow Cytometry/ Cytogenetics	X			X		
Chimerism				X	X	X
MRD monitoring (3) *				X	X	X
Concomitant Diseases and treatment	X	X	X	X	X	X

Adverse Events			X	X	X	X

(1) To be checked daily during treatment
(2) CMV PCR and Cyclosporin levels will be checked twice a week or more often if apropriate during and after treatment as part of our standard care for transplant patients
(3) If MRD marker available (e.g bcr-abl)

*These tests are performed for safety reasons only. Data will not be collected for analysis of the study.

5 PATIENT SELECTION

5.1 Treatment Group

Patients fulfilling the eligibility criteria (section 5.3) who:

- Have any of the following:

1) Refractory AML
2) Relapsed AML
3) MDS – Intermediate 2 or High Risk group (IPSS 1.5 or grater)
4) Other High Risk Myeloid Malignancy
5)

AND

- Are deemed unsuitable or decline a myeloablative transplant

5.2 Patient Registration

Patients must be entered and registered with the Investigator. The patient's eligibility will be checked at patient registration and actual laboratory measurements will be requested. If eligible for the study, the patient will be allocated a Patient Number by the Investigator.

Before registering the patient the Investigator or designated representative should determine the eligibility of the patient. Where there is a doubt as the eligibility, the Investigator should consult with the Chief Investigator. If the agreed opinion is that the patient is potentially eligible, the patient will be registered.

5.3 Eligibility Criteria

5.2.1 Inclusion Criteria

1. Diagnosis of histologically documented AML (any WHO type), with primary induction failure, or at relapse where the patient is not a candidate or does not wish to proceed to a myeloablative transplant. Also, histologically / cytogenetically documented diagnosis of MDS (IPSS Int. 2, HR) , or other high risk Myeloid Malignancy where the patient is not a candidate or does not wish to proceed to a myeloablative transplant.

2. All acute toxic effects of any prior radiotherapy, chemotherapy, or surgical procedures must have resolved to National Cancer Institute (NCI) Common Toxicity Criteria (CTC)(Version 3.0) Grade < 2 (with the exception of chemotherapy-induced alopecia). Surgery must have occurred at least 21 days prior to initiation of treatment.

3. Aged between 18 and 60 years old (inclusive).

4. Last dose of antineoplastic therapy must be more than 14 days from starting treatment, except for hydroxyurea or Low Dose Cytarabinewhich may have been administered up to 24 hours prior to first study drug administration for leukoreduction.

5. ECOG performance status must be 0, 1, or 2.

6. Life expectancy of at least 2 months.

7. Pregnancy test (females of childbearing potential) Negative

8. Signed informed consent indicating that they are aware of the neoplastic nature of their disease and have been informed of the procedures to be followed, alternatives, potential benefits, side effects, risks, and discomforts.

9. Willing and able to comply with scheduled visits, treatment plan, and laboratory tests.

10. If sexually active, male and female patients must agree that both they and their partner will employ an effective method of birth control throughout the active study.

5.3.2 Exclusion Criteria

1. Concurrent therapy with any other investigational agent.

2. Pregnant or breastfeeding women. All at-risk female subjects must have a negative pregnancy test within 10 days prior to the start of treatment.

3. Clinically significant cardiac disease (New York Heart Association, Class III or IV)

4. Dementia or altered mental status that would prohibit informed consent.

5. Other severe, acute, or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the subject inappropriate for this study.

6. Current malignancies at other sites, with the exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin. Cancer survivors, who have undergone potentially curative therapy for a prior malignancy, have no evidence of that disease for five years and are deemed at low risk for recurrence, are eligible for the study.

6 INVESTIGATIONS SCHEDULE

Please also refer to the tabulated Schedule of Assessments in Section 3.6.

In sections 6.1 - 6.3 procedures in *italic* font are for patient safety only and will not be recorded or analysed as part of this trial.

6.1 Pre-treatment Evaluations

The following must be performed **within 4 weeks** of the patient starting treatment:

3. Aged between 18 and 60 years old (inclusive).

4. Last dose of antineoplastic therapy must be more than 14 days from starting treatment, except for hydroxyurea or Low Dose Cytarabinewhich may have been administered up to 24 hours prior to first study drug administration for leukoreduction.

5. ECOG performance status must be 0, 1, or 2.

6. Life expectancy of at least 2 months.

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6.1 Pre-treatment Evaluations

The following must be performed **within 4 weeks** of the patient starting treatment:

6.3 Assessment of Disease

- Bone Marrow examination at Days 30 and when clinically indicated
- *MRD monitoring, if applicable*

7 <u>PHARMACOVIGILANCE PROCEDURES</u>

7.1 Adverse Events

Adverse events will not be recorded on the CRF, unless they meet the criteria of a SAE (See Section 7.2) or are unexpected.

Expected Adverse Events are:

- Nausea and vomiting
- Alopecia
- Mucositis
- Diarrhoea
- Infertility
- Bone marrow suppression with cytopenias
- Blood product support
- Haemorrhagic complications
- Neutropenic infections (often accompanied by hypertension, renal impairment, DIC, multi-organ failure and requirement for intensive care)
- Fungal infections
- Peri-engratment syndrome
- Graft versus Host Disease (GVHD)
- Opportunistic infections
- Death due to relapsed / refractory disease
- Expected adverse reactions associated with medication given in this protocol (see section 7.3.2)

7.2 General Definitions – Serious Adverse Events (SAEs)

7.2.1 Serious Adverse Events

A Serious Adverse Event (SAE) is defined in general as an untoward (unfavourable) event, which is:

- fatal or life-threatening,
- requires or prolongs hospitalisation,
- is significantly or permanently disabling or incapacitating,
- constitutes a congenital anomaly or a birth defect or
- may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

7.2.2 Suspected Unexpected Serious Adverse Reactions (SUSARs)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is a serious adverse drug reaction which also demonstrates the following characteristic of being unexpected:

Unexpected – An adverse event, the nature **OR** severity of which is **NOT** consistent with the applicable Summary of Medicinal Product Characteristics.

7.3 Operational Definition - Serious Adverse Events (SAEs)

7.3.1 Events not classed as SAEs

The following events **will not** be reportable as SAEs within this trial:
Hospitalisation, or prolongation of hospitalisation for:

- Routine treatment or monitoring of the studied indications not associated with any deterioration in condition.
- Treatment which was elective or pre-planned, for a pre-existing condition not associated with any deterioration in condition.
- Admission to hospital or other institution for general care, not associated with any deterioration in condition.
- Treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions for serious as given above and not resulting in hospital admission.

It is also well recognized that adverse events that may be life threatening are a normal consequence of Acute Myeloid Leukemia or indeed any other of the myeloid malignancies we plan to treat on this study and of their effective treatments. Therefore, the following will not require to be reported as SAEs

- Neutropenic infections
- Fungal Infections
- Blood product support
- Haemorrhagic complications
- Graft versus host disease
- Opportunistic infections
- Any Grade 3 or 4 haematologic toxicity
- Death due to refractory or relapsed disease

7.3.2 Expected SARs

The following events **WILL BE** classed as expected SARs within this trial and therefore will **NOT** be reportable as SAEs or SUSARs:

All investigators should refer to the Summary of Medicinal Product Characteristics (SmPC) for each IMP when determining whether a SAR is expected.

The following table lists some of the side effects/potential ARs listed in the SMPCs for the protocol-specified IMPs. These therefore should be considered as expected events and thus would not meet the criteria of an unexpected reaction. This table should be used as a guide only and the most recent and relevant SMPC must be referred to for more specific details and potential drug interactions.

Drug / Treatment	Example of Expected Adverse Reactions
IMPs	
Cyclophosphamide	Cystitis or haematuria Mucositis Alopecia Bone marrow suppression Infertility Cardio-toxicity
Fludarabine	Bone Marrow Suppression Opportunistic infections

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Drug / Treatment	Example of Expected Adverse Reactions
IMPs	
Cyclophosphamide	Cystitis or haematuria Mucositis Alopecia Bone marrow suppression Infertility Cardio-toxicity
Fludarabine	Bone Marrow Suppression Opportunistic infections

All SAEs occurring whilst on the trial (until 30 days after the last day of the last treatment cycle for patients receiving chemotherapy or until 100 days post transplant for patients receiving stem cell transplant) must be recorded on the CRF.

SUSARS

All SAEs considered by the Chief Investigator to be both suspected to be related to protocol-treatment and unexpected will be classified as SUSARs and will be subject to expedited reporting to the MHRA. The Chief Investigator or Co-Investigator will, within 24 hours of learning of the SUSAR, fax an initial SUSAR report to the Sponsor and to the Main REC. All SUSARs occurring whilst on trial (until 30 days after the last day of the last treatment cycle for patients receiving chemotherapy or until 100 days post transplant for patients receiving stem cell transplant) must be recorded on the CRF.

Fax Number for reporting SUSARs:
0207 882 5958

7.4.2 Data Items

For each SAE, the following information will be collected:

- full details in medical terms with a diagnosis, if possible
- its duration (start and end dates; times, if applicable)
- action taken
- outcome
- causality, in the opinion of the investigator*
- whether the event would be considered expected or unexpected (see Section 7.4)*

*Assessment of causality and expectedness for trials involving IMPs must be made by an authorised medic. If an authorised medic is unavailable, initial reports without causality and expectedness assessment should be submitted to the Sponsor by a healthcare professional within 24 hours, but must be followed up by medical assessment as soon as possible thereafter.

Any follow-up information should be faxed to the Sponsor as soon as it is available. Events will be followed up until the event has resolved or a final outcome has been reached.

7.5 Operational Definition of Treatment Related Mortality (TRM)

Treatment Related Mortality is defined as any death within the first 100 days after stem cell transplantation or within one month of the last chemotherapy treatment not due to other causes. Treatment Related Mortality is assessed for each death occurring whilst a patient is on the trial. Each death is recorded on the CRF; where the death is suspected to be related to the trial treatment, a completed SAE report form is to be faxed to the Sponsor within 24 hours of notification to the research team.

Fax Number for reporting TRDs:
0207 882 5958

8 EFFICACY

The efficacy of this approach will be assessed through the various end points of the study as described in section 2.2.

We aim to demonstrate a 40% 2year Overall Survival as that would confirm existing data on the efficacy of this approach.

9 PATIENT WITHDRAWAL

The Investigator will make every reasonable effort to keep each patient on study. However, if the Investigator removes a patient from the study or if the patient declines further participation, prior to any therapeutic intervention, final assessments will be performed, if possible.

Patients who are removed from the study due to adverse experiences (clinical or laboratory) will be treated and followed according to accepted medical practice

The following are justifiable reasons for the Investigator to withdraw a patient from study:

- unacceptable toxicity
- unforeseen events: any event which in the judgement of the Investigator makes further treatment inadvisable
- SAE requiring discontinuation of treatment
- withdrawal of consent - where patient is not evaluable, additional patients will be recruited to replace them
- serious violation of the study protocol
- withdrawal by the Investigator for clinical reasons not related to the study.
- evidence of disease progression

10 DISCONTINUATION OF STUDY

The study will terminate when:

- The stated number of patients to be recruited is reached.
- The stated objectives of the trial are achieved
- At any point during the study if unacceptable toxicities are observed.
  The mortality rate will be monitored by calculating 95% Confidence Intervals (CIs) for survival at 100 days. If the CIs show that there is significant probability that there is a greater than 25% non-relapse mortality rate at day 100, then the study will be stopped.

11. <u>DATA ANALYSIS</u>

11.1 Statistical Considerations

We aim to demonstrate a 40% 2year Overall Survival as that would confirm existing data on the efficacy of this approach. With that in mind, we aim to recruit 93 patients in this study as a sample of that size can give us the anticipated outcome with a 95% Confidence Interval of 0.1.
These sample size has been calculated using the following formula:

$N= [P(1-P)]/(WIDTH/1.96)^2$

Where, P=proportion (in this case 0.40), WIDTH is the width of the confidence interval, and N is the sample size

11.2 Toxicities / Mortality

The mortality rate will be monitored by calculating 95% Confidence Intervals for survival at 100 days. If the CIs show that there is significant probability that the survival rate is less then 75% at day 100, then the study will be stopped.

11.3 Efficacy

This will be assessed by the various primary and secondary endpoints.

We aim to demonstrate a 40% 2year Overall Survival, as that would confirm existing data on the efficacy of this approach.

12. <u>DATA HANDLING AND RECORD KEEPING</u>

All information collected during the course of the study will be kept strictly confidential and the 1998 Data Protection Act will be complied with.

12.1 Study Documents

As this is a single site study, all documentation will be kept in the Trial Master File, according to Centre for Experimental Cancer Medicine (CECM) SOPs.

12.2 Case Report Forms

A Case Report Form (CRF) is required for each individual patient. CRFs will be completed according to the CRF guidelines.

12.3 Record Retention and Archiving

At the end of the trial, all essential documents, as defined by GCP, will be archived for a minimum of 20 years. During this time, documents must remain available for inspection by the sponsor or auditors. Following written authorisation from the sponsor, arrangements for confidential destruction will be made.

In the Chief Investigator withdraws from the study, all responsibilities should be transferred to a designee.

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13 GOVERNANCE ISSUES

To ensure the responsibility and accountability for the overall quality of care received by patients during the study period, clinical governance issues pertaining to all aspects of routine management will be bought to the attention of the CI and Sponsor.

13.1 Monitoring

This is a single site study and no Monitoring Plan exists. The CECM Self Monitoring Form will be completed every 6 months, as a minimum, by a member of the Coordinating Team. Copies of all reports will be forwarded to the JRO Governance Team.

13.2 Audit and Inspection

The study may be audited by representatives from the coordinating centre and sponsor. Additionally, inspections may also be carrier out by the Competent Authority. The Investigator will be informed of the audit outcome. Investigators are obliged to cooperate in any inspection allowing the auditor or inspector direct access to all relevant documents and allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings. Audit and inspection may occur at any time during or after the completion of the study.

13.3 Serious breaches in GCP or trial protocol

The Chief Investigator will promptly notify the Sponsor of a serious breach (as defined in Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 [Statutory Instrument 2004/1031], as amended by Statutory Instrument 2006/1928) that they become aware of. A "serious breach" is a breach which is likely to effect to a significant degree:-

- The safety or physical or mental integrity of the subjects of the trial; or
- The scientific value of the trial.

13.4 Ethical Considerations

This study will be conducted in compliance with the protocol, the International Conference on Harmonisation of Good Clinical Practice (ICH GCP) guidelines and the Declaration of Helsinki (Edinburgh, 2000).

13.5 Data Monitoring Committee (DMC)

Membership of the DMC is outlined in the DMC Charter.

The primary purpose of the DMC is to monitor safety during the trial. The DMC will review TRM data on an ongoing basis and report to the Chief Investigator.

14 SPONSORSHIP AND INDEMINTY

Dr. Jamie Cavenagh at Barts and The London NHS Trust is the Chief Investigator.
Barts and The London NHS Trust is also sponsoring the study.

15 PUBLICATION POLICY

This is an Investigator-led study sponsored by Barts and The London NHS Trust. The data collected will not be used to license/register any pharmaceuticals. Authorship of the final manuscript(s), interim publications, or abstracts will be decided according to active participation in the statistical design, accrual of eligible patients and statistical analysis. Participating Investigators will be acknowledged in the final manuscript, and the correct designation for this site is 'Centre for Experimental Cancer Medicine, Barts Cancer Institute, Queen Mary University of London'. Representatives for the Sponsor will be added, as appropriate, as co-authors.

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